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(54) Title: NOVEL DNA CLONING METHOD (57) Abstract <p>The invention refers to a novel method for cloning DNA molecules using a homologous recombination mechanism between at least two DNA molecules comprising: a) providing a host cell capable of performing homologous recombination, b) contacting in said host cell a first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred.</p>		

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Novel DNA cloning method

Description

5 The invention refers to a novel method for cloning DNA molecules using a homologous recombination mechanism between at least two DNA molecules. Further, novel reagent kits suitable for DNA cloning are provided.

Current methods for cloning foreign DNA in bacterial cells usually comprise
10 the steps of providing a suitable bacterial vector, cleaving said vector with a restriction enzyme and in vitro-inserting a foreign DNA fragment in said vector. The resulting recombinant vectors are then used to transform bacteria. Although such cloning methods have been used successfully for about 20 years they suffer from several drawbacks. These drawbacks are,
15 in particular, that the in vitro steps required for inserting foreign DNA in a vector are often very complicated and time-consuming, if no suitable restriction sites are available on the foreign DNA or the vector.

Furthermore, current methods usually rely on the presence of suitable
20 restriction enzyme cleavage sites in the vector into which the foreign DNA fragment is placed. This imposes two limitations on the final cloning product. First, the foreign DNA fragment can usually only be inserted into the vector at the position of such a restriction site or sites. Thus, the cloning product is limited by the disposition of suitable restriction sites and
25 cloning into regions of the vector where there is no suitable restriction site, is difficult and often imprecise. Second, since restriction sites are typically 4 to 8 base pairs in length, they occur a multiple number of times as the size of the DNA molecules being used increases. This represents a practical limitation to the size of the DNA molecules that can be manipulated by most
30 current cloning techniques. In particular, the larger sizes of DNA cloned into vectors such as cosmids, BACs, PACs and P1s are such that it is usually impractical to manipulate them directly by restriction enzyme based

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techniques. Therefore, there is a need for providing a new cloning method, from which the drawbacks of the prior art have at least partly been eliminated.

- 5 According to the present invention it was found that an efficient homologous recombination mechanism between two DNA molecules occurs at usable frequencies in a bacterial host cell which is capable of expressing the products of the *recE* and *recT* genes or functionally related genes such as the *red α* and *red β* genes, or the phage P22 recombination system
- 10 (Kolodner et al., Mol.Microbiol. 11 (1994) 23-30; Fenton, A.C. and Poteete, A.R., Virology 134 (1984) 148-160; Poteete, A.R. and Fenton, A.C., Virology 134 (1984) 161-167). This novel method of cloning DNA fragments is termed "ET cloning".
- 15 The identification and characterization of the E.coli RecE and RecT proteins is described Gillen et al. (J.Bacteriol. 145 (1981), 521-532) and Hall et al. (J.Bacteriol. 175 (1993), 277-287). Hall and Kolodner (Proc.Natl.Acad.Sci. USA 91 (1994), 3205-3209) disclose in vitro homologous pairing and strand exchange of linear double-stranded DNA and homologous circular
- 20 single-stranded DNA promoted by the RecT protein. Any references to the use of this method for the cloning of DNA molecules in cells cannot be found therein.
- The *recET* pathway of genetic recombination in E.coli is known (Hall and
- 25 Kolodner (1994), supra; Gillen et al. (1981), supra). This pathway requires the expression of two genes, *recE* and *recT*. The DNA sequence of these genes has been published (Hall et al., supra). The RecE protein is similar to bacteriophage proteins, such as λ *exo* or λ *Red α* (Gillen et al., J.Mol.Biol. 113 (1977), 27-41; Little, J.Biol.Chem. 242 (1967), 679-686;
- 30 Radding and Carter, J.Biol.Chem. 246 (1971), 2513-2518; Joseph and Kolodner, J.Biol.Chem. 258 (1983), 10418-10424). The RecT protein is similar to bacteriophage proteins, such as λ *β* -protein or λ *Red β* (Hall et al.

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(1993), *supra*; Muniyappa and Radding, J.Biol.Chem. 261 (1986), 7472-7478; Kmiec and Hollomon, J.Biol.Chem.256 (1981), 12636-12639). The content of the above-cited documents is incorporated herein by reference.

5 Oliner et al. (Nucl.Acids Res. 21 (1993), 5192-5197) describe in vivo cloning of PCR products in E.coli by intermolecular homologous recombination between a linear PCR product and a linearized plasmid vector. Other previous attempts to develop new cloning methods based on homologous recombination in prokaryotes, too, relied on the use of
10 restriction enzymes to linearise the vector (Bubeck et al., Nucleic Acids Res. 21 (1993), 3601-3602; Oliner et al., Nucleic Acids Res. 21 (1993), 5192-5197; Degryse, Gene 170 (1996), 45-50) or on the host-specific recA-dependent recombination system (Hamilton et al., J.Bacteriol. 171 (1989), 4617-4622; Yang et al., Nature Biotech. 15 (1997), 859-865; Dabert and
15 Smith, Genetics 145 (1997), 877-889). These methods are of very limited applicability and are hardly used in practice.

The novel method of cloning DNA according to the present invention does not require in vitro treatments with restriction enzymes or DNA ligases and
20 is therefore fundamentally distinct from the standard methodologies of DNA cloning. The method relies on a pathway of homologous recombination in E.coli involving the recE and recT gene products, or the red α and red β gene products, or functionally equivalent gene products. The method covalently combines one preferably linear and preferably extrachromosomal DNA
25 fragment, the DNA fragment to be cloned, with one second preferably circular DNA vector molecule, either an episome or the endogenous host chromosome or chromosomes. It is therefore distinct from previous descriptions of cloning in E.coli by homologous recombination which either rely on the use of two linear DNA fragments or different recombination
30 pathways.

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The present invention provides a flexible way to use homologous recombination to engineer large DNA molecules including an intact > 76 kb plasmid and the E.coli chromosome. Thus, there is practically no limitation of target choice either according to size or site. Therefore, any recipient DNA in a host cell, from high copy plasmid to the genome, is amenable to precise alteration. In addition to engineering large DNA molecules, the invention outlines new, restriction enzyme-independent approaches to DNA design. For example, deletions between any two chosen base pairs in a target episome can be made by choice of oligonucleotide homology arms. Similarly, chosen DNA sequences can be inserted at a chosen base pair to create, for example, altered protein reading frames. Concerted combinations of insertions and deletions, as well as point mutations, are also possible. The application of these strategies is particularly relevant to complex or difficult DNA constructions, for example, those intended for homologous recombinations in eukaryotic cells, e.g. mouse embryonic stem cells. Further, the present invention provides a simple way to position site specific recombination target sites exactly where desired. This will simplify applications of site specific recombination in other living systems, such as plants and mice.

A subject matter of the present invention is a method for cloning DNA molecules in cells comprising the steps:

- a) providing a host cell capable of performing homologous recombination,
- b) contacting in said host cell a first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and
- c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred.

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In the method of the present invention the homologous recombination preferably occurs via the recET mechanism, i.e. the homologous recombination is mediated by the gene products of the recE and the recT genes which are preferably selected from the E.coli genes recE and recT or functionally related genes such as the phage λ red α and red β genes.

The host cell suitable for the method of the present invention preferably is a bacterial cell, e.g. a gram-negative bacterial cell. More preferably, the host cell is an enterobacterial cell, such as Salmonella, Klebsiella or Escherichia. Most preferably the host cell is an Escherichia coli cell. It should be noted, however, that the cloning method of the present invention is also suitable for eukaryotic cells, such as fungi, plant or animal cells.

Preferably, the host cell used for homologous recombination and propagation of the cloned DNA can be any cell, e.g. a bacterial strain in which the products of the recE and recT, or red α and red β , genes are expressed. The host cell may comprise the recE and recT genes located on the host cell chromosome or on non-chromosomal DNA, preferably on a vector, e.g. a plasmid. In a preferred case, the RecE and RecT, or Red α and Red β , gene products are expressed from two different regulatable promoters, such as the arabinose-inducible BAD promoter or the lac promoter or from non-regulatable promoters. Alternatively, the recE and recT, or red α and red β , genes are expressed on a polycistronic mRNA from a single regulatable or non-regulatable promoter. Preferably the expression is controlled by regulatable promoters.

Especially preferred is also an embodiment, wherein the recE or red α gene is expressed by a regulatable promoter. Thus, the recombinogenic potential of the system is only elicited when required and, at other times, possible undesired recombination reactions are limited. The recT or red β gene, on the other hand, is preferably overexpressed with respect to recE or red α . This may be accomplished by using a strong constitutive promoter, e.g. the

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EM7 promoter and/or by using a higher copy number of *recT*, or *red β* , versus *recE*, or *red α* , genes.

For the purpose of the present invention any *recE* and *recT* genes are
5 suitable insofar as they allow a homologous recombination of first and
second DNA molecules with sufficient efficiency to give rise to
recombination products in more than 1 in 10⁹ cells transfected with DNA.
The *recE* and *recT* genes may be derived from any bacterial strain or from
bacteriophages or may be mutants and variants thereof. Preferred are *recE*
10 and *recT* genes which are derived from *E.coli* or from *E.coli* bacteriophages,
such as the *red α* and *red β* genes from lambdoid phages, e.g. bacteriophage
 λ .

More preferably, the *recE* or *red α* gene is selected from a nucleic acid
15 molecule comprising

- (a) the nucleic acid sequence from position 1320 (ATG) to 2159 (GAC) as depicted in Fig.7B,
- (b) the nucleic acid sequence from position 1320 (ATG) to 1998(CGA) as depicted in Fig.14B,
- 20 (c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
- (d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequence from (a), (b) and/or (c).

25 More preferably, the *recT* or *red β* gene is selected from a nucleic acid molecule comprising

- (a) the nucleic acid sequence from position 2155 (ATG) to 2961 (GAA) as depicted in Fig.7B,
- (b) the nucleic acid sequence from position 2086 (ATG) to 2868 (GCA) as
30 depicted in Fig.14B,
- (c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or

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(d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequences from (a), (b) and/or (c).

It should be noted that the present invention also encompasses mutants and variants of the given sequences, e.g. naturally occurring mutants and variants or mutants and variants obtained by genetic engineering. Further it should be noted that the *recE* gene depicted in Fig.7B is an already truncated gene encoding amino acids 588-866 of the native protein. Mutants and variants preferably have a nucleotide sequence identity of at least 60%, preferably of at least 70% and more preferably of at least 80% of the *recE* and *recT* sequences depicted in Fig.7B and 13B, and of the *red α* and *red β* sequences depicted in Fig.14B.

According to the present invention hybridization under stringent conditions preferably is defined according to Sambrook et al. (1989), *infra*, and comprises a detectable hybridization signal after washing for 30 min in 0.1 x SSC, 0.5% SDS at 55°C, preferably at 62°C and more preferably at 68°C.

In a preferred case the *recE* and *recT* genes are derived from the corresponding endogenous genes present in the *E.coli* K12 strain and its derivatives or from bacteriophages. In particular, strains that carry the *sbcA* mutation are suitable. Examples of such strains are JC8679 and JC 9604 (Gillen et al. (1981), *supra*). Alternatively, the corresponding genes may also be obtained from other coliphages such as lambdoid phages or phage P22.

The genotype of JC 8679 and JC 9604 is Sex (Hfr, F+, F-, or F') : F-. JC 8679 comprises the mutations: *recBC* 21, *recC* 22, *sbcA* 23, *thr*-1, *ara*-14, *leu* B 6, *DE* (*gpt-proA*) 62, *lacY*1, *tsx*-33, *gluV*44 (AS), *galK*2 (Oc), LAM-, *his*-60, *relA* 1, *rps* L31 (*strR*), *xyl* A5, *mtl*-1, *argE*3 (Oc) and *thi*-1. JC 9604 comprises the same mutations and further the mutation *recA* 56.

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Further, it should be noted that the *recE* and *recT*, or *red α* and *red β* , genes can be isolated from a first donor source, e.g. a donor bacterial cell and transformed into a second receptor source, e.g. a receptor bacterial or eukaryotic cell in which they are expressed by recombinant DNA means.

5

In one embodiment of the invention, the host cell used is a bacterial strain having an *sbcA* mutation, e.g. one of *E.coli* strains JC 8679 and JC 9604 mentioned above. However, the method of the invention is not limited to host cells having an *sbcA* mutation or analogous cells. Surprisingly, it has been found that the cloning method of the invention also works in cells without *sbcA* mutation, whether *recBC* + or *recBC* -, e.g. also in prokaryotic *recBC* + host cells, e.g. in *E.coli* *recBC* + cells. In that case preferably those host cells are used in which the product of a *recBC* type exonuclease inhibitor gene is expressed. Preferably, the exonuclease inhibitor is capable of inhibiting the host *recBC* system or an equivalent thereof. A suitable example of such exonuclease inhibitor gene is the λ *red γ* gene (Murphy, J.Bacteriol. 173 (1991), 5808-5821) and functional equivalents thereof, respectively, which, for example, can be obtained from other coliphages such as from phage P22 (Murphy, J.Biol.Chem.269 (1994), 22507-22516).

20

More preferably, the exonuclease inhibitor gene is selected from a nucleic acid molecule comprising

(a) the nucleic acid sequence from position 3588 (ATG) to 4002 (GTA) as depicted in Fig.14A,

25 (b) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or

(c) a nucleic acid sequence which hybridizes under stringent conditions (as defined above) with the nucleic acid sequence from (a) and/ or (b).

30

Surprisingly, it has been found that the expression of an exonuclease inhibitor gene in both *recBC* + and *recBC* - strains leads to significant improvement of cloning efficiency.

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The cloning method according to the present invention employs a homologous recombination between a first DNA molecule and a second DNA molecule. The first DNA molecule can be any DNA molecule that carries an origin of replication which is operative in the host cell, e.g. an E.coli replication origin. Further, the first DNA molecule is present in a form which is capable of being replicated in the host cell. The first DNA molecule, i.e. the vector, can be any extrachromosomal DNA molecule containing an origin of replication which is operative in said host cell, e.g. a plasmid including single, low, medium or high copy plasmids or other extrachromosomal circular DNA molecules based on cosmid, P1, BAC or PAC vector technology. Examples of such vectors are described, for example, by Sambrook et al. (Molecular Cloning, Laboratory Manual, 2nd Edition (1989), Cold Spring Harbor Laboratory Press) and Ioannou et al. (Nature Genet. 6 (1994), 84-89) or references cited therein. The first DNA molecule can also be a host cell chromosome, particularly the E.coli chromosome. Preferably, the first DNA molecule is a double-stranded DNA molecule.

The second DNA molecule is preferably a linear DNA molecule and comprises at least two regions of sequence homology, preferably of sequence identity to regions on the first DNA molecule. These homology or identity regions are preferably at least 15 nucleotides each, more preferably at least 20 nucleotides and, most preferably, at least 30 nucleotides each. Especially good results were obtained when using sequence homology regions having a length of about 40 or more nucleotides, e.g. 60 or more nucleotides. The two sequence homology regions can be located on the linear DNA fragment so that one is at one end and the other is at the other end, however they may also be located internally. Preferably, also the second DNA molecule is a double-stranded DNA molecule.

30

The two sequence homology regions are chosen according to the experimental design. There are no limitations on which regions of the first

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DNA molecule can be chosen for the two sequence homology regions located on the second DNA molecule, except that the homologous recombination event cannot delete the origin of replication of the first DNA molecule. The sequence homology regions can be interrupted by non-identical sequence regions as long as sufficient sequence homology is retained for the homologous recombination reaction. By using sequence homology arms having non-identical sequence regions compared to the target site mutations such as substitutions, e.g. point mutations, insertions and/or deletions may be introduced into the target site by ET cloning.

The second foreign DNA molecule which is to be cloned in the bacterial cell may be derived from any source. For example, the second DNA molecule may be synthesized by a nucleic acid amplification reaction such as a PCR where both of the DNA oligonucleotides used to prime the amplification contain in addition to sequences at the 3'-ends that serve as a primer for the amplification, one or the other of the two homology regions. Using oligonucleotides of this design, the DNA product of the amplification can be any DNA sequence suitable for amplification and will additionally have a sequence homology region at each end.

A specific example of the generation of the second DNA molecule is the amplification of a gene that serves to convey a phenotypic difference to the bacterial host cells, in particular, antibiotic resistance. A simple variation of this procedure involves the use of oligonucleotides that include other sequences in addition to the PCR primer sequence and the sequence homology region. A further simple variation is the use of more than two amplification primers to generate the amplification product. A further simple variation is the use of more than one amplification reaction to generate the amplification product. A further variation is the use of DNA fragments obtained by methods other than PCR, for example, by endonuclease or restriction enzyme cleavage to linearize fragments from any source of DNA.

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It should be noted that the second DNA molecule is not necessarily a single species of DNA molecule. It is of course possible to use a heterogenous population of second DNA molecules, e.g. to generate a DNA library, such as a genomic or cDNA library.

5

The method of the present invention may comprise the contacting of the first and second DNA molecules in vivo. In one embodiment of the present invention the second DNA fragment is transformed into a bacterial strain that already harbors the first vector DNA molecule. In a different
10 embodiment, the second DNA molecule and the first DNA molecule are mixed together in vitro before co-transformation in the bacterial host cell. These two embodiments of the present invention are schematically depicted in Fig.1. The method of transformation can be any method known in the art (e.g. Sambrook et al. supra). The preferred method of transformation or co-
15 transformation, however, is electroporation.

After contacting the first and second DNA molecules under conditions which favour homologous recombination between first and second DNA molecules via the ET cloning mechanism a host cell is selected, in which
20 homologous recombination between said first and second DNA molecules has occurred. This selection procedure can be carried out by several different methods. In the following three preferred selection methods are depicted in Fig.2 and described in detail below.

25 In a first selection method a second DNA fragment is employed which carries a gene for a marker placed between the two regions of sequence homology wherein homologous recombination is detectable by expression of the marker gene. The marker gene may be a gene for a phenotypic marker which is not expressed in the host or from the first DNA molecule.
30 Upon recombination by ET cloning, the change in phenotype of the host strain conveyed by the stable acquisition of the second DNA fragment identifies the ET cloning product.

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In a preferred case, the phenotypic marker is a gene that conveys resistance to an antibiotic, in particular, genes that convey resistance to kanamycin, ampicillin, chloramphenicol, tetracyclin or any other substance that shows bacteriocidal or bacteriostatic effects on the bacterial strain employed.

5

A simple variation is the use of a gene that complements a deficiency present within the bacterial host strain employed. For example, the host strain may be mutated so that it is incapable of growth without a metabolic supplement. In the absence of this supplement, a gene on the second DNA
10 fragment can complement the mutational defect thus permitting growth. Only those cells which contain the episome carrying the intended DNA rearrangement caused by the ET cloning step will grow.

In another example, the host strain carries a phenotypic marker gene which
15 is mutated so that one of its codons is a stop codon that truncates the open reading frame. Expression of the full length protein from this phenotypic marker gene requires the introduction of a suppressor tRNA gene which, once expressed, recognizes the stop codon and permits translation of the full open reading frame. The suppressor tRNA gene is introduced by the ET
20 cloning step and successful recombinants identified by selection for, or identification of, the expression of the phenotypic marker gene. In these cases, only those cells which contain the intended DNA rearrangement caused by the ET cloning step will grow.

25 A further simple variation is the use of a reporter gene that conveys a readily detectable change in colony colour or morphology. In a preferred case, the green fluorescence protein (GFP) can be used and colonies carrying the ET cloning product identified by the fluorescence emissions of GFP. In another preferred case, the lacZ gene can be used and colonies
30 carrying the ET cloning product identified by a blue colony colour when X-gal is added to the culture medium.

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In a second selection method the insertion of the second DNA fragment into the first DNA molecule by ET cloning alters the expression of a marker present on the first DNA molecule. In this embodiment the first DNA molecule contains at least one marker gene between the two regions of sequence homology and homologous recombination may be detected by an altered expression, e.g. lack of expression of the marker gene.

In a preferred application, the marker present on the first DNA molecule is a counter-selectable gene product, such as the *sacB*, *ccdB* or tetracycline-resistance genes. In these cases, bacterial cells that carry the first DNA molecule unmodified by the ET cloning step after transformation with the second DNA fragment, or co-transformation with the second DNA fragment and the first DNA molecule, are plated onto a medium so the expression of the counter-selectable marker conveys a toxic or bacteriostatic effect on the host. Only those bacterial cells which contain the first DNA molecule carrying the intended DNA rearrangement caused by the ET cloning step will grow.

In another preferred application, the first DNA molecule carries a reporter gene that conveys a readily detectable change in colony colour or morphology. In a preferred case, the green fluorescence protein (GFP) can be present on the first DNA molecule and colonies carrying the first DNA molecule with or without the ET cloning product can be distinguished by differences in the fluorescence emissions of GFP. In another preferred case, the *lacZ* gene can be present on the first DNA molecule and colonies carrying the first DNA molecule with or without the ET cloning product identified by a blue or white colony colour when X-gal is added to the culture medium.

In a third selection method the integration of the second DNA fragment into the first DNA molecule by ET cloning removes a target site for a site specific recombinase, termed here an RT (for recombinase target) present

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on the first DNA molecule between the two regions of sequence homology. A homologous recombination event may be detected by removal of the target site.

5 In the absence of the ET cloning product, the RT is available for use by the corresponding site specific recombinase. The difference between the presence or not of this RT is the basis for selection of the ET cloning product. In the presence of this RT and the corresponding site specific recombinase, the site specific recombinase mediates recombination at this
10 RT and changes the phenotype of the host so that it is either not able to grow or presents a readily observable phenotype. In the absence of this RT, the corresponding site specific recombinase is not able to mediate recombination.

15 In a preferred case, the first DNA molecule to which the second DNA fragment is directed, contains two RTs, one of which is adjacent to, but not part of, an antibiotic resistance gene. The second DNA fragment is directed, by design, to remove this RT. Upon exposure to the corresponding site specific recombinase, those first DNA molecules that do not carry the ET
20 cloning product will be subject to a site specific recombination reaction between the RTs that remove the antibiotic resistance gene and therefore the first DNA molecule fails to convey resistance to the corresponding antibiotic. Only those first DNA molecules that contain the ET cloning product, or have failed to be site specifically recombined for some other
25 reason, will convey resistance to the antibiotic.

In another preferred case, the RT to be removed by ET cloning of the second DNA fragment is adjacent to a gene that complements a deficiency present within the host strain employed. In another preferred case, the RT
30 to be removed by ET cloning of the second DNA fragment is adjacent to a reporter gene that conveys a readily detectable change in colony colour or morphology.

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In another preferred case, the RT to be removed by ET cloning of the second DNA fragment is anywhere on a first episomal DNA molecule and the episome carries an origin of replication incompatible with survival of the bacterial host cell if it is integrated into the host genome. In this case the host genome carries a second RT, which may or may not be a mutated RT so that the corresponding site specific recombinase can integrate the episome, via its RT, into the RT sited in the host genome. Other preferred RTs include RTs for site specific recombinases of the resolvase/transposase class. RTs include those described from existing examples of site specific recombination as well as natural or mutated variations thereof.

The preferred site specific recombinases include Cre, FLP, Kw or any site specific recombinase of the integrase class. Other preferred site specific recombinases include site specific recombinases of the resolvase/transposase class.

There are no limitations on the method of expression of the site specific recombinase in the host cell. In a preferred method, the expression of the site specific recombinase is regulated so that expression can be induced and quenched according to the optimisation of the ET cloning efficiency. In this case, the site specific recombinase gene can be either integrated into the host genome or carried on an episome. In another preferred case, the site specific recombinase is expressed from an episome that carries a conditional origin of replication so that it can be eliminated from the host cell.

In another preferred case, at least two of the above three selection methods are combined. A particularly preferred case involves a two-step use of the first selection method above, followed by use of the second selection method. This combined use requires, most simply, that the DNA fragment to be cloned includes a gene, or genes that permits the identification, in the first step, of correct ET cloning products by the acquisition of a phenotypic

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change. In a second step, expression of the gene or genes introduced in the first step is altered so that a second round of ET cloning products can be identified. In a preferred example, the gene employed is the tetracycline resistance gene and the first step ET cloning products are identified by the acquisition of tetracycline resistance. In the second step, loss of expression of the tetracycline gene is identified by loss of sensitivity to nickel chloride, fusaric acid or any other agent that is toxic to the host cell when the tetracycline gene is expressed. This two-step procedure permits the identification of ET cloning products by first the integration of a gene that conveys a phenotypic change on the host, and second by the loss of a related phenotypic change, most simply by removal of some of the DNA sequences integrated in the first step. Thereby the genes used to identify ET cloning products can be inserted and then removed to leave ET cloning products that are free of these genes.

In a further embodiment of the present invention the ET cloning may also be used for a recombination method comprising the steps of

- a) providing a source of RecE and RecT, or Red α and Red β , proteins,
- b) contacting a first DNA molecule which is capable of being replicated in a suitable host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and
- c) selecting DNA molecules in which a homologous recombination between said first and second DNA molecules has occurred.

The source of RecE and RecT, or Red α and Red β , proteins may be either purified or partially purified RecE and RecT, or Red α and Red β , proteins or cell extracts comprising RecE and RecT, or Red α and Red β , proteins.

The homologous recombination event in this embodiment may occur in vitro, e.g. when providing a cell extract containing further components

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required for homologous recombination. The homologous recombination event, however, may also occur in vivo, e.g. by introducing RecE and RecT, or Red α and Red β , proteins or the extract in a host cell (which may be recET positive or not, or red $\alpha\beta$ positive or not) and contacting the DNA molecules in the host cell. When the recombination occurs in vitro the selection of DNA molecules may be accomplished by transforming the recombination mixture in a suitable host cell and selecting for positive clones as described above. When the recombination occurs in vivo the selection methods as described above may directly be applied.

A further subject matter of the invention is the use of cells, preferably bacterial cells, most preferably, E.coli cells capable of expressing the recE and recT, or red α and red β , genes as a host cell for a cloning method involving homologous recombination.

Still a further subject matter of the invention is a vector system capable of expressing recE and recT, or red α and red β , genes in a host cell and its use for a cloning method involving homologous recombination. Preferably, the vector system is also capable of expressing an exonuclease inhibitor gene as defined above, e.g. the λ red γ gene. The vector system may comprise at least one vector. The recE and recT, or red α and red β , genes are preferably located on a single vector and more preferably under control of a regulatable promoter which may be the same for both genes or a single promoter for each gene. Especially preferred is a vector system which is capable of overexpressing the recT, or red β , gene versus the recE, or red α , gene.

Still a further subject matter of the invention is the use of a source of RecE and RecT, or Red α and Red β , proteins for a cloning method involving homologous recombination.

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A still further subject matter of the invention is a reagent kit for cloning comprising

- (a) a host cell, preferably a bacterial host cell,
- (b) means of expressing *recE* and *recT*, or *red α* and *red β* , genes in
5 said host cell, e.g. comprising a vector system, and
- (c) a recipient cloning vehicle, e.g. a vector, capable of being replicated in said cell.

10 On the one hand, the recipient cloning vehicle which corresponds to the first DNA molecule of the process of the invention can already be present in the bacterial cell. On the other hand, it can be present separated from the bacterial cell.

In a further embodiment the reagent kit comprises

- 15 (a) a source for *RecE* and *RecT*, or *Red α* and *Red β* , proteins and
- (b) a recipient cloning vehicle capable of being propagated in a host cell and
- (c) optionally a host cell suitable for propagating said recipient cloning vehicle.

20 The reagent kit furthermore contains, preferably, means for expressing a site specific recombinase in said host cell, in particular, when the recipient ET cloning product contains at least one site specific recombinase target site. Moreover, the reagent kit can also contain DNA molecules suitable for use as a source of linear DNA fragments used for ET cloning, preferably by

25 serving as templates for PCR generation of the linear fragment, also as specifically designed DNA vectors from which the linear DNA fragment is released by restriction enzyme cleavage, or as prepared linear fragments included in the kit for use as positive controls or other tasks. Moreover, the reagent kit can also contain nucleic acid amplification primers comprising

30 a region of homology to said vector. Preferably, this region of homology is located at the 5'-end of the nucleic acid amplification primer.

The invention is further illustrated by the following Sequence listings, Figures and Examples.

- SEQ ID NO. 1: shows the nucleic acid sequence of the plasmid pBAD24-rec ET (Fig. 7).
- 5 SEQ ID NOs 2/3: show the nucleic acid and amino acid sequences of the truncated recE gene (t-recE) present on pBAD24-recET at positions 1320-2162.
- SEQ ID NOs 4/5: show the nucleic acid and amino acid sequences of the recT gene present on pBAD24-recET at position 2155-2972.
- 10 SEQ ID NOs 6/7: show the nucleic acid and amino acid sequences of the araC gene present on the complementary stand to the one shown of pBAD24-recET at positions 974-996.
- SEQ ID NOs 8/9: show the nucleic acid and amino acid sequences of the bla gene present on pBAD24-recET at positions 3493-4353.
- 15 SEQ ID NO 10: shows the nucleic acid sequence of the plasmid pBAD-ET γ (Fig. 13).
- SEQ ID No 11: shows the nucleic acid sequence of the plasmid pBAD- $\alpha\beta\gamma$ (Fig. 14) as well as the coding regions for the genes red α (1320-200), red β (2086-2871) and red γ (3403-3819).
- 20 SEQ ID NOs 12-14: show the amino acid sequences of the Red α , Red β and Red γ proteins, respectively. The red γ sequence is present on each of pBAD-ET γ (Fig. 13) and pBAD- $\alpha\beta\gamma$ (Fig. 14).
- 25

Figure 1

A preferred method for ET cloning is shown by diagram. The linear DNA fragment to be cloned is synthesized by PCR using oligonucleotide primers that contain a left homology arm chosen to match sequences in the recipient episome and a sequence for priming in the PCR reaction, and a

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right homology arm chosen to match another sequence in the recipient episome and a sequence for priming in the PCR reaction. The product of the PCR reaction, here a selectable marker gene (sm1), is consequently flanked by the left and right homology arms and can be mixed together in vitro with the episome before co-transformation, or transformed into a host cell harboring the target episome. The host cell contains the products of the recE and recT genes. ET cloning products are identified by the combination of two selectable markers, sm1 and sm2 on the recipient episome.

Figure 2

Three ways to identify ET cloning products are depicted. The first, (on the left of the figure), shows the acquisition, by ET cloning, of a gene that conveys a phenotypic difference to the host, here a selectable marker gene (sm). The second (in the centre of the figure) shows the loss, by ET cloning, of a gene that conveys a phenotypic difference to the host, here a counter selectable marker gene (counter-sm). The third shows the loss of a target site (RT, shown as triangles on the circular episome) for a site specific recombinase (SSR), by ET cloning. In this case, the correct ET cloning product deletes one of the target sites required by the SSR to delete a selectable marker gene (sm). The failure of the SSR to delete the sm gene identifies the correct ET cloning product.

Figure 3

A simple example of ET cloning is presented.

(a) Top panel - PCR products (left lane) synthesized from oligonucleotides designed as described in Fig.1 to amplify by PCR a kanamycin resistance gene and to be flanked by homology arms present in the recipient vector, were mixed in vitro with the recipient vector (2nd lane) and cotransformed into a recET+ E.coli host. The recipient vector carried an ampicillin

resistance gene. (b) Transformation of the *sbca* E.coli strain JC9604 with either the PCR product alone (0.2 μ g) or the vector alone (0.3 μ g) did not convey resistance to double selection with ampicillin and kanamycin (amp + kan), however cotransformation of both the PCR product and the

5 vector produced double resistant colonies. More than 95% of these colonies contained the correct ET cloning product where the kanamycin gene had precisely integrated into the recipient vector according to the choice of homology arms. The two lanes on the right of (a) show Pvu II restriction enzyme digestion of the recipient vector before and after ET cloning. (c) As

10 for b, except that six PCR products (0.2 μ g each) were cotransformed with pSVpaZ11 (0.3 μ g each) into JC9604 and plated onto Amp + Kan plates or Amp plates. Results are plotted as Amp + Kan-resistant colonies, representing recombination products, divided by Amp-resistant colonies, representing the plasmid transformation efficiency of the competent cell

15 preparation, $\times 10^6$. The PCR products were equivalent to the a-b PCR product except that homology arm lengths were varied. Results are from five experiments that used the same batches of competent cells and DNAs. Error bars represent standard deviation. (d) Eight products flanked by 50 bp homology arms were cotransformed with pSVpaZ11 into JC9604. All eight

20 PCR products contained the same left homology arm and amplified neo gene. The right homology arms were chosen from the pSVpaZ11 sequence to be adjacent to (0), or at increasing distances (7-3100 bp), from the left. Results are from four experiments.

25

Figure 4

ET cloning in an approximately 100kb P1 vector to exchange the selectable marker.

30 A P1 clone which uses a kanamycin resistance gene as selectable marker and which contains at least 70kb of the mouse Hox a gene cluster was used. Before ET cloning, this episome conveys kanamycin resistance (top

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panel, upper left) to its host *E. coli* which are ampicillin sensitive (top panel, upper right). A linear DNA fragment designed to replace the kanamycin resistance gene with an ampicillin resistance gene was made by PCR as outlined in Fig. 1 and transformed into *E. coli* host cells in which the recipient
5 Hox a/P1 vector was resident. ET cloning resulted in the deletion of the kanamycin resistance gene, and restoration of kanamycin sensitivity (top panel, lower left) and the acquisition of ampicillin resistance (top panel, lower right). Precise DNA recombination was verified by restriction digestion and Southern blotting analyses of isolated DNA before and after ET cloning
10 (lower panel).

Figure 5

ET cloning to remove a counter selectable marker
15 A PCR fragment (upper panel, left, third lane) made as outlined in Figs. 1 and 2 to contain the kanamycin resistance gene was directed by its chosen homology arms to delete the counter selectable *ccdB* gene present in the vector, pZero-2.1. The PCR product and the pZero vector were mixed in vitro (upper panel, left, 1st lane) before cotransformation into a *recE/recT* +
20 *E. coli* host. Transformation of pZero-2.1 alone and plating onto kanamycin selection medium resulted in little colony growth (lower panel, left). Cotransformation of pZero-2.1 and the PCR product presented ET cloning products (lower panel, right) which showed the intended molecular event as visualized by *Pvu* II digestion (upper panel, right).

25

Figure 6

ET cloning mediated by inducible expression of *recE* and *recT* from an episome.
RecE/RecT mediate homologous recombination between linear and circular
30 DNA molecules. (a) The plasmid pBAD24-*recET* was transformed into *E. coli* JC5547, and then batches of competent cells were prepared after induction of RecE/RecT expression by addition of L-arabinose for the times indicated

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before harvesting. A PCR product, made using oligonucleotides e and f to contain the chloramphenicol resistance gene (cm) of pMAK705 and 50 bp homology arms chosen to flank the ampicillin resistance gene (bla) of pBAD24-recET, was then transformed and recombinants identified on chloramphenicol plates. (b) Arabinose was added to cultures of pBAD24-recET transformed JC5547 for different times immediately before harvesting for competent cell preparation. Total protein expression was analyzed by SDS-PAGE and Coomassie blue staining. (c) The number of chloramphenicol resistant colonies per μg of PCR product was normalized against a control for transformation efficiency, determined by including 5 pg pZero2.1, conveying kanamycin resistance, in the transformation and plating an aliquot onto Kan plates.

Figure 7A

The plasmid pBAD24-recET is shown by diagram. The plasmid contains the genes recE (in a truncated form) and recT under control of the inducible BAD promoter (P_{BAD}). The plasmid further contains an ampicillin resistance gene (Amp^r) and an araC gene.

Figure 7B

The nucleic acid sequence and the protein coding portions of pBAD24-recET are depicted.

Figure 8

Manipulation of a large E.coli episome by multiple recombination steps. a Scheme of the recombination reactions. A P1 clone of the Mouse Hoxa complex, resident in JC9604, was modified by recombination with PCR products that contained the neo gene and two Flp recombination targets

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(FRTs). The two PCR products were identical except that one was flanked by g and h homology arms (insertion), and the other was flanked by i and h homology arms (deletion). In a second step, the neo gene was removed by Flp recombination between the FRTs by transient transformation of a Flp expression plasmid based on the pSC101 temperature-sensitive origin (ts ori). b Upper panel; ethidium bromide stained agarose gel showing EcoR1 digestions of P1 DNA preparations from three independent colonies for each step. Middle panel; a Southern blot of the upper panel hybridized with a neo gene probe. Lower panel; a Southern blot of the upper panel hybridized with a Hoxa3 probe to visualize the site of recombination. Lanes 1, the original Hoxa3 P1 clone grown in E.coli strain NS3145. Lanes 2, replacement of the Tn903 kanamycin resistance gene resident in the P1 vector with an ampicillin resistance gene increased the 8.1 kb band (lanes 1), to 9.0 kb. Lanes 3, insertion of the Tn5-neo gene with g-h homology arms upstream of Hoxa3, increased the 6.7 kb band (lanes 1,2) to 9.0 kb. Lanes 4, Flp recombinase deleted the g-h neo gene reducing the 9.0 kb band (lanes 3) back to 6.7 kb. Lanes 5, deletion of 6 kb of Hoxa3 - 4 intergenic DNA by replacement with the i-h neo gene, decreased the 6.7 kb band (lanes 2) to 4.5 kb. Lanes 6, Flp recombinase deleted the i-h neo gene reducing the 4.5 kb band to 2.3 kb.

Figure 9

Manipulation of the E.coli chromosome. A Scheme of the recombination reactions. The endogenous lacZ gene of JC9604 at 7.8' of the E.coli chromosome, shown in expanded form with relevant Ava I sites and coordinates, was targeted by a PCR fragment that contained the neo gene flanked by homology arms j and k, and loxP sites, as depicted. Integration of the neo gene removed most of the lacZ gene including an Ava I site to alter the 1443 and 3027 bp bands into a 3277 bp band. In a second step, the neo gene was removed by Cre recombination between the loxPs by transient transformation of a Cre expression plasmid based on the pSC101

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temperature-sensitive origin (ts ori). Removal of the neo gene by Cre recombinase reduces the 3277 band to 2111 bp. b β -galactosidase expression evaluated by streaking colonies on X-Gal plates. The top row of three streaks show β -galactosidase expression in the host JC9604 strain (w.t.), the lower three rows (Km) show 24 independent primary colonies, 20 of which display a loss of β -galactosidase expression indicative of the intended recombination event. c Southern analysis of E.coli chromosomal DNA digested with Ava I using a random primed probe made from the entire lacZ coding region; lanes 1,2, w.t.; lanes 3-6, four independent white colonies after integration of the j-k neo gene; lanes 7-10; the same four colonies after transient transformation with the Cre expression plasmid.

Figure 10

Two rounds of ET cloning to introduce a point mutation. a Scheme of the recombination reactions. The lacZ gene of pSVpaX1 was disrupted in JC9604lacZ, a strain made by the experiment of Fig.9 to ablate endogenous lacZ expression and remove competitive sequences, by a sacB-neo gene cassette, synthesized by PCR to pIB279 and flanked by l and m homology arms. The recombinants, termed pSV-sacB-neo, were selected on Amp + Kan plates. The lacZ gene of pSV-sacB-neo was then repaired by a PCR fragment made from the intact lacZ gene using l' and m' homology arms. The m' homology arm included a silent C to G change that created a BamH1 site. The recombinants, termed pSVpaX1', were identified by counter selection against the sacB gene using 7% sucrose. b β -galactosidase expression from pSVpaX1 was disrupted in pSV-sacB-neo and restored in pSVpaX1'. Expression was analyzed on X-gal plates. Three independent colonies of each pSV-sacB-neo and pSVpaX1' are shown. c Ethidium bromide stained agarose gels of BamH1 digested DNA prepared from independent colonies taken after counter selection with sucrose. All β -galactosidase expressing colonies (blue) contained the introduced BamH1 restriction site (upper panel). All white colonies displayed large

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rearrangements and no product carried the diagnostic 1.5kb BamH1 restriction fragment (lower panel).

Figure 11

5

Transference of ET cloning into a *recBC* + host to modify a large episome. a Scheme of the plasmid, pBAD-ET γ , which carries the mobile ET system, and the strategy employed to target the Hoxa P1 episome. pBAD-ET γ is based on pBAD24 and includes (i) the truncated *recE* gene (t-*recE*) under the arabinose-inducible P_{BAD} promoter; (ii) the *recT* gene under the EM7 promoter; and (iii) the *red γ* gene under the Tn5 promoter. It was transformed into NS3145, a *recA* E.coli strain which contained the Hoxa P1 episome. After arabinose induction, competent cells were prepared and transformed with a PCR product carrying the chloramphenicol resistance gene (cm) flanked by n and p homology arms. n and p were chosen to recombine with a segment of the P1 vector. b Southern blots of Pvu II digested DNAs hybridized with a probe made from the P1 vector to visualize the recombination target site (upper panel) and a probe made from the chloramphenicol resistance gene (lower panel). Lane 1, DNA prepared from cells harboring the Hoxa P1 episome before ET cloning. Lanes 2-17, DNA prepared from 16 independent chloramphenicol resistant colonies.

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15
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Figure 12

Comparison of ET cloning using the *recE/recT* genes in pBAD-ET γ with *red α /red β* genes in pBAD- $\alpha\beta\gamma$.

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The plasmids pBAD-ET γ or pBAD- $\alpha\beta\gamma$, depicted, were transformed into the E.coli *recA*-, *recBC* + strain, DK1 and targeted by a chloramphenicol gene as described in Fig.6 to evaluate ET cloning efficiencies. Arabinose induction of protein expression was for 1 hour.

30

Figure 13A

The plasmid pBAD-ET γ is shown by diagram.

5 **Figure 13B**

The nucleic acid sequence and the protein coding portions of pBAD-ET γ are depicted.

10 **Figure 14A**

The plasmid pBAD- $\alpha\beta\gamma$ is shown by diagram. This plasmid substantially corresponds to the plasmid shown in Fig.13 except that the recE and recT genes are substituted by the red α and red β genes.

15

Figure 14B

The nucleic acid sequence and the protein coding portions of pBAD- $\alpha\beta\gamma$ are depicted.

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1. Methods**1.1. Preparation of linear fragments**

Standard PCR reaction conditions were used to amplify linear DNA
25 fragments. The sequences of the primers used are depicted in Table 1.

Table 1

30 The Tn5-neo gene from pJP5603 (Penfold and Pemberton, Gene 118 (1992), 145-146) was amplified by using oligo pairs a/b and c/d. The chloramphenicol (cm) resistant gene from pMAK705 (Hashimoto-Gotoh and

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Sekiguchi, J. *Bacteriol.* 131 (1977), 405-412) was amplified by using primer pairs e/f and n/p. The Tn5-neo gene flanked by FRT or loxP sites was amplified from pKaZ or pKaX (<http://www.embl-heidelberg.de/ExternalInfo/stewart>) using oligo pairs i/h, g/h and j/k. The sacB-neo cassette from plB279 (Blomfield et al., *Mol. Microbiol.* 5 (1991), 1447-1457) was amplified by using oligo pair l/m. The lacZ gene fragment from pSVpaZ11 (Buchholz et al., *Nucleic Acids Res.* 24 (1996), 4256-4262) was amplified using oligo pair l'/m'. PCR products were purified using the QIAGEN PCR Purification Kit and eluted with H₂O₂, followed by digestion of any residual template DNA with Dpn I. After digestion, PCR products were extracted once with Phenol:CHCl₃, ethanol precipitated and resuspended in H₂O at approximately 0.5 µg/µl.

1.2 Preparation of competent cells and electroporation

Saturated overnight cultures were diluted 50 fold into LB medium, grown to an OD600 of 0.5, following by chilling on ice for 15 min. Bacterial cells were centrifuged at 7,000 rpm for 10 min at 0°C. The pellet was resuspended in ice-cold 10% glycerol and centrifuged again (7,000 rpm, -5°C, 10 min). This was repeated twice more and the cell pellet was suspended in an equal volume of ice-cold 10% glycerol. Aliquots of 50 µl were frozen in liquid nitrogen and stored at -80°C. Cells were thawed on ice and 1 µl DNA solution (containing, for co-transformation, 0.3 µg plasmid and 0.2 µg PCR products; or, for transformation, 0.2 µg PCR products) was added. Electroporation was performed using ice-cold cuvettes and a Bio-Rad Gene Pulser set to 25 µFD, 2.3 kV with Pulse Controller set at 200 ohms. LB medium (1 ml) was added after electroporation. The cells were incubated at 37°C for 1 hour with shaking and then spread on antibiotic plates.

1.3 Induction of RecE and RecT expression

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E.coli JC5547 carrying pBAD24-recET was cultured overnight in LB medium plus 0.2% glucose, 100 μ g/ml ampicillin. Five parallel LB cultures, one of which (0) included 0.2% glucose, were started by a 1/100 inoculation. The cultures were incubated at 37°C with shaking for 4 hours and 0.1% L-arabinose was added 3, 2, 1 or 1/2 hour before harvesting and processing as above. Immediately before harvesting, 100 μ l was removed for analysis on a 10% SDS-polyacrylamide gel. E.coli NS3145 carrying Hoxa-P1 and pBAD-ET γ was induced by 0.1% L-arabinose for 90 min before harvesting.

10

1.4 Transient transformation of FLP and Cre expression plasmids

The FLP and Cre expression plasmids, 705-Cre and 705-FLP (Buchholz et al, Nucleic Acids Res. 24 (1996), 3118-3119), based on the pSC101 temperature sensitive origin, were transformed into rubidium chloride competent bacterial cells. Cells were spread on 25 μ g/ml chloramphenicol plates, and grown for 2 days at 30°C, whereupon colonies were picked, replated on L-agar plates without any antibiotics and incubated at 40°C overnight. Single colonies were analyzed on various antibiotic plates and all showed the expected loss of chloramphenicol and kanamycin resistance.

20

1.5 Sucrose counter selection of sacB expression

The E.coli JC9604lacZ strain, generated as described in Fig.11, was cotransformed with a sacB-neo PCR fragment and pSVpaX1 (Buchholz et al, Nucleic Acids Res. 24 (1996), 4256-4262). After selection on 100 μ g/ml ampicillin, 50 μ g/ml kanamycin plates, pSVpaX-sacB-neo plasmids were isolated and cotransformed into fresh JC9604lacZ cells with a PCR fragment amplified from pSVpaX1 using primers l'/m'. Oligo m' carried a silent point mutation which generated a BamHI site. Cells were plated on 7% sucrose, 100 μ g/ml ampicillin, 40 μ g/ml X-gal plates and incubated at

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- 30 -

28°C for 2 days. The blue and white colonies grown on sucrose plates were counted and further checked by restriction analysis.

1.6 Other methods

5

DNA preparation and Southern analysis were performed according to standard procedures. Hybridization probes were generated by random priming of fragments isolated from the Tn5 neo gene (PvuII), Hoxa3 gene (both HindIII fragments), lacZ genes (EcoRI and BamHI fragments from pSVpaX1), cm gene (BstBI fragments from pMAK705) and P1 vector fragments (2.2 kb EcoRI fragments from P1 vector).

10

2. Results

15

2.1 Identification of recombination events in E.coli

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To identify a flexible homologous recombination reaction in E.coli, an assay based on recombination between linear and circular DNAs was designed (Fig. 1, Fig. 3). Linear DNA carrying the Tn5 kanamycin resistance gene (neo) was made by PCR (Fig. 3a). Initially, the oligonucleotides used for PCR amplification of neo were 60mers consisting of 42 nucleotides at their 5' ends identical to chosen regions in the plasmid and, at the 3' ends, 18 nucleotides to serve as PCR primers. Linear and circular DNAs were mixed in equimolar proportions and co-transformed into a variety of E.coli hosts. Homologous recombination was only detected in sbcA E.coli hosts. More than 95% of double ampicillin/kanamycin resistant colonies (Fig. 3b) contained the expected homologously recombined plasmid as determined by restriction digestion and sequencing. Only a low background of kanamycin resistance, due to genomic integration of the neo gene, was apparent (not shown).

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The linear plus circular recombination reaction was characterized in two ways. The relationship between homology arm length and recombination efficiency was simple, with longer arms recombining more efficiently (Fig.3c). Efficiency increased within the range tested, up to 60 bp. The effect of distance between the two chosen homology sites in the recipient plasmid was examined (Fig.3d). A set of eight PCR fragments was generated by use of a constant left homology arm with differing right homology arms. The right homology arms were chosen from the plasmid sequence to be 0 - 3100 bp from the left. Correct products were readily obtained from all, with less than 4 fold difference between them, although the insertional product (0) was least efficient. Correct products also depended on the presence of both homology arms, since PCR fragments containing only one arm failed to work.

2.2 Involvement of RecE and RecT

The relationship between host genotype and this homologous recombination reaction was more systematically examined using a panel of E.coli strains deficient in various recombination components (Table 2).

Table 2

Only the two sbcA strains, JC8679 and JC9604 presented the intended recombination products and RecA was not required. In sbcA strains, expression of RecE and RecT is activated. Dependence on recE can be inferred from comparison of JC8679 with JC8691. Notably no recombination products were observed in JC9387 suggesting that the sbcBC background is not capable of supporting homologous recombination based on 50 nucleotide homology arms.

To demonstrate that RecE and RecT are involved, part of the recET operon was cloned into an inducible expression vector to create pBAD24-recET

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(Fig.6a). the recE gene was truncated at its N-terminal end, as the first 588 a.a.s of RecE are dispensable. The recBC strain, JC5547, was transformed with pBAD24-recET and a time course of RecE/RecT induction performed by adding arabinose to the culture media at various times before harvesting for competent cells. The batches of harvested competent cells were evaluated for protein expression by gel electrophoresis (Fig.6b) and for recombination between a linear DNA fragment and the endogenous pBAD24-recET plasmid (Fig.6c). Without induction of RecE/RecT, no recombinant products were found, whereas recombination increased in approximate concordance with increased RecE/RecT expression. This experiment also shows that co-transformation of linear and circular DNAs is not essential and the circular recipient can be endogenous in the host. From the results shown in Figs.3, 6 and Table 2, we conclude that RecE and RecT mediate a very useful homologous recombination reaction in recBC E.coli at workable frequencies. Since RecE and RecT are involved, we refer to this way of recombining linear and circular DNA fragments as "ET cloning".

2.3 Application of ET cloning to large target DNAs

20

To show that large DNA episomes could be manipulated in E.coli, a > 76 kb P1 clone that contains at least 59 kb of the intact mouse Hoxa complex, (confirmed by DNA sequencing and Southern blotting), was transferred to an E.coli strain having an sbcA background (JC9604) and subjected to two rounds of ET cloning. In the first round, the Tn903 kanamycin resistance gene resident in the P1 vector was replaced by an ampicillin resistance gene (Fig.4). In the second round, the interval between the Hoxa3 and a4 genes was targeted either by inserting the neo gene between two base pairs upstream of the Hoxa3 proximal promoter, or by deleting 6203 bp between the Hoxa3 and a4 genes (Fig.8a). Both insertional and deletional ET cloning products were readily obtained (Fig.8b, lanes 2, 3 and 5) showing that the

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two rounds of ET cloning took place in this large E.coli episome with precision and no apparent unintended recombination.

5 The general applicability of ET cloning was further examined by targeting a gene in the E.coli chromosome (Fig.9a). The β -galactosidase (lacZ) gene of JC9604 was chosen so that the ratio between correct and incorrect recombinants could be determined by evaluating β -galactosidase expression. Standard conditions (0.2 μ g PCR fragment; 50 μ l competent cells), produced 24 primary colonies, 20 of which were correct as
10 determined by β -galactosidase expression (Fig.9b), and DNA analysis (Fig.9c, lanes 3-6).

2.4 Secondary recombination reactions to remove operational sequences

15 The products of ET cloning as described above are limited by the necessary inclusion of selectable marker genes. Two different ways to use a further recombination step to remove this limitation were developed. In the first way, site specific recombination mediated by either Flp or Cre recombinase was employed. In the experiments of Figs.8 and 9, either Flp recombination
20 target sites (FRTs) or Cre recombination target sites (loxPs) were included to flank the neo gene in the linear substrates. Recombination between the FRTs or loxPs was accomplished by Flp or Cre, respectively, expressed from plasmids with the pSC101 temperature sensitive replication origin (Hashimoto-Gotoh and Sekiguchi, J.Bacteriol. 131 (1977), 405-412) to
25 permit simple elimination of these plasmids after site specific recombination by temperature shift. The precisely recombined Hoxa P1 vector was recovered after both ET and Flp recombination with no other recombination products apparent (Fig.8, lanes 4 and 6). Similarly, Cre recombinase precisely recombined the targeted lacZ allele (Fig.9, lanes 7-10). Thus site
30 specific recombination can be readily coupled with ET cloning to remove operational sequences and leave a 34 bp site specific recombination target site at the point of DNA manipulation.

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In the second way to remove the selectable marker gene, two rounds of ET cloning, combining positive and counter selection steps, were used to leave the DNA product free of any operational sequences (Fig.10a).

5 Additionally this experiment was designed to evaluate, by a functional test based on β -galactosidase activity, whether ET cloning promoted small mutations such as frame shift or point mutations within the region being manipulated. In the first round, the lacZ gene of pSVpaX1 was disrupted with a 3.3 kb PCR fragment carrying the neo and B.subtilis sacB (Blomfield
10 et al., Mol.Microbiol. 5 (1991), 1447-1457) genes, by selection for kanamycin resistance (Fig.10a). As shown above for other positively selected recombination products, virtually all selected colonies were white (Fig.10b), indicative of successful lacZ disruption, and 17 of 17 were confirmed as correct recombinants by DNA analysis. In the second round,
15 a 1.5 kb PCR fragment designed to repair lacZ was introduced by counter selection against the sacB gene. Repair of lacZ included a silent point mutation to create a BamH1 restriction site. Approximately one quarter of sucrose resistant colonies expressed β -galactosidase, and all analyzed (17 of 17; Fig.10c) carried the repaired lacZ gene with the BamH1 point
20 mutation. The remaining three quarters of sucrose resistant colonies did not express β -galactosidase, and all analyzed (17 of 17; Fig.10c) had undergone a variety of large mutational events, none of which resembled the ET cloning product. Thus, in two rounds of ET cloning directed at the lacZ gene, no disturbances of β -galactosidase activity by small mutations
25 were observed, indicating the RecE/RecT recombination works with high fidelity. The significant presence of incorrect products observed in the counter selection step is an inherent limitation of the use of counter selection, since any mutation that ablates expression of the counter selection gene will be selected. Notably, all incorrect products were large
30 mutations and therefore easily distinguished from the correct ET product by DNA analysis. In a different experiment (Fig.5), we observed that ET cloning into pZero2.1 (InVitroGen) by counter selection against the ccdB gene gave

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a lower background of incorrect products (8%), indicating that the counter selection background is variable according to parameters that differ from those that influence ET cloning efficiencies.

5 2.5 Transference of ET cloning between E.coli hosts

The experiments shown above were performed in recBC- E.coli hosts since the sbcA mutation had been identified as a suppressor of recBC (Barbour et al., Proc.Natl.Acad.Sci. USA 67 (1970), 128-135; Clark, Genetics 78
10 (1974), 259-271). However, many useful E.coli strains are recBC+, including strains commonly used for propagation of P1, BAC or PAC episomes. To transfer ET cloning into recBC+ strains, we developed pBAD-ET γ and pBAD- $\alpha\beta\gamma$ (Figs.13 and 14). These plasmids incorporate three features important to the mobility of ET cloning. First, RecBC is the major
15 E.coli exonuclease and degrades introduced linear fragments. Therefore the RecBC inhibitor, Red γ (Murphy, J.Bacteriol. 173 (1991), 5808-5821), was included. Second, the recombinogenic potential of RecE/RecT, or Red α /Red β , was regulated by placing recE or red α under an inducible promoter. Consequently ET cloning can be induced when required and
20 undesired recombination events which are restricted at other times. Third, we observed that ET cloning efficiencies are enhanced when RecT, or Red β , but not RecE, or Red α , is overexpressed. Therefore we placed recT, or red β , under the strong, constitutive, EM7 promoter.

25 pBAD-ET γ was transformed into NS3145 E.coli harboring the original Hoxa P1 episome (Fig.11a). A region in the P1 vector backbone was targeted by PCR amplification of the chloramphenicol resistance gene (cm) flanked by n and p homology arms. As described above for positively selected ET cloning reactions, most (> 90%) chloramphenicol resistant colonies were
30 correct. Notably, the overall efficiency of ET cloning, in terms of linear DNA transformed, was nearly three times better using pBAD-ET γ than with similar experiments based on targeting the same episome in the sbcA host,

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JC9604. This is consistent with our observation that overexpression of RecT improves ET cloning efficiencies.

5 A comparison between ET cloning efficiencies mediated by RecE/RecT, expressed from pBAD-ET γ , and Red α /Red β , expressed from pBAD- $\alpha\beta\gamma$ was made in the recA $^{-}$, recBC $^{+}$ E.coli strain, DK1 (Fig.12). After transformation of E.coli DK1 with either pBAD-ET γ or pBAD- $\alpha\beta\gamma$, the same experiment as described in Figure 6a,c, to replace the bla gene of the pBAD vector with a chloramphenicol gene was performed. Both pBAD-ET γ or pBAD- $\alpha\beta\gamma$
10 presented similar ET cloning efficiencies in terms of responsiveness to arabinose induction of RecE and Red α , and number of targeted events.

Table 2

E.coli Strains	Genotypes	Amp+Kan	Amp
			$\times 10^8/\mu\text{g}$
JC8679	<i>recBC sbcA</i>	318	2.30
JC9604	<i>recA recBC sbcA</i>	114	0.30
JC8691	<i>recBC sbcA recE</i>	0	0.37
JC5547	<i>recA recBC</i>	0	0.37
JC5519	<i>recBC</i>	0	1.80
JC15329	<i>recA recBC sbcBC</i>	0	0.03
JC9387	<i>recBC sbcBC</i>	0	2.20
JC8111	<i>recBC sbcBC recF</i>	0	2.40
JC9366	<i>recA</i>	0	0.37
JC13031	<i>recJ</i>	0	0.45

Claims

1. A method for cloning DNA molecules in cells comprising the steps of:
 - a) providing a host cell capable of performing homologous recombination,
 - b) contacting in said host cell a first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and
 - c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred.
2. The method according to claim 1 wherein the homologous recombination occurs via the recET cloning mechanism.
3. The method according to claim 2 wherein the host cell is capable of expressing recE and recT genes.
4. The method according to claim 3 wherein the recE and recT genes are selected from E.coli recE and recT genes or from λ red α and red β genes.
5. The method according to claim 3 or 4 wherein the host cell is transformed with at least one vector capable of expressing recE and/or recT genes.
6. The method of claim 3, 4 or 5 wherein the expression of the recE and/or recT genes is under control of a regulatable promoter.

7. The method of claim 5 or 6 wherein the recT gene is overexpressed versus the recE gene.
8. The method according to any one of claims 3 to 7 wherein the recE gene is selected from a nucleic acid molecule comprising
- 5 (a) the nucleic acid sequence from position 1320 (ATG) to 2159 (GAC) as depicted in Fig.7B,
- (b) the nucleic acid sequence from position 1320 (ATG) to 1998 (CGA) as depicted in Fig.13B,
- 10 (c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
- (d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequence from (a), (b) and/or (c).
9. The method according to any one of claims 3 to 8 wherein the recT gene is selected from a nucleic acid molecule comprising
- 15 (a) the nucleic acid sequence from position 2155 (ATG) to 2961 (GAA) as depicted in Fig.7B,
- (b) the nucleic acid sequence from position 2086 (ATG) to 2868 (GCA) as depicted in Fig.13B,
- 20 (c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
- (d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequences from (a), (b) and/or (c).
- 25 10. The method according to any one of the previous claims wherein the host cell is a gram-negative bacterial cell.
11. The method according to claim 10 wherein the host cell is an Escherichia coli cell.
- 30

12. The method according to claim 11 wherein the host cell is an Escherichia coli K12 strain.
13. The method according to claim 12 wherein the E.coli strain is
5 selected from JC 8679 and JC 9604.
14. The method according to any one of the previous claims wherein the host cell further is capable of expressing a recBC inhibitor gene.
- 10 15. The method according to claim 14 wherein the host cell is transformed with a vector expressing the recBC inhibitor gene.
16. The method according to claim 14 or 15 wherein the recBC inhibitor gene is selected from a nucleic acid molecule comprising
15 (a) the nucleic acid sequence from position 3588 (ATG) to 4002 (GTA) as depicted in Fig.13B,
(b) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
(c) a nucleic acid sequence which hybridizes under stringent
20 conditions (as defined above) with the nucleic acid sequence from (a) and/ or (b).
17. The method according to any one of claims 13 to 16 wherein the host cell is a prokaryotic recBC+ cell.
25
18. The method according to any one of the previous claims wherein the first DNA molecule is circular.
19. The method according to any one of the previous claims wherein the
30 first DNA molecule is an extrachromosomal DNA molecule containing an origin of replication which is operative in the host cell.

20. The method according to claim 18 or 19 wherein the first DNA molecule is selected from plasmids, cosmids, P1 vectors, BAC vectors and PAC vectors.
- 5 21. The method according to any one of claims 1-18 wherein the first DNA molecule is a host cell chromosome.
22. The method according to any one of the previous claims wherein the second DNA molecule is linear.
- 10 23. The method according to any one of the previous claims wherein the regions of sequence homology are at least 15 nucleotides each.
- 15 24. The method according to one of claims 1 to 16 wherein the second DNA molecule is obtained by an amplification reaction.
25. The method according to one of the previous claims wherein the first and/or second DNA molecules are introduced into the host cells by transformation.
- 20 26. The method according to claim 25 wherein the transformation method is electroporation.
- 25 27. The method according to one of claims 1 to 26 wherein the first and second DNA molecules are introduced into the host cell simultaneously by co-transformation.
- 30 28. The method according to one of claims 1 to 26 wherein the second DNA molecule is introduced into a host cell in which the first DNA molecule is already present.

29. The method according to one of the previous claims wherein the second DNA molecule contains at least one marker gene placed between the two regions of sequence homology and wherein homologous recombination is detected by expression of said marker gene.
30. The method according to claim 29 wherein gene presence is selected from antibiotic resistance genes, deficiency complementation genes and reporter genes.
31. The method of any one of claims 1 to 30 wherein the first DNA molecule contains at least one marker gene between the two regions of sequence homology and wherein homologous recombination is detected by lack of expression of said marker gene.
32. The method of any one of claims 1 to 31 wherein said marker gene is selected from genes which, under selected conditions, convey a toxic or bacteriostatic effect on the cell, and reporter genes.
33. A method according to any one of the previous claims wherein the first DNA molecule contains at least one target site for a site specific recombinase between the two regions of sequence homology and wherein homologous recombination is detected by removal of said target site.
34. A method for cloning DNA molecules comprising the steps of:
- (a) providing a source of RecE and RecT proteins,
 - (b) contacting a first DNA molecule which is capable of being replicated in a suitable host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and

(c) selecting DNA molecules in which homologous recombination between said first and second DNA molecules has occurred.

- 5 35. The method of claim 34 wherein said RecE and RecT or proteins are selected from E.coli RecE and RecT proteins or from phage λ Red α and Red β proteins.
- 10 36. The method of claim 34 or 35 wherein the recombination occurs in vitro.
37. The method of claim 34 or 35 wherein the recombination occurs in vivo.
- 15 38. Use of cells capable of expressing the recE and recT genes as a host cell for a cloning method involving homologous recombination.
39. Use of a vector system capable of expressing recE and recT genes in a host cell for a cloning method involving homologous recombination.
- 20 40. Use of claims 38 or 39 wherein the recE and recT genes are selected from E.coli recE and recT genes or from λ red α and red β genes.
41. Use of a source of RecE and RecT proteins for a cloning method involving homologous recombination.
- 25 42. Use of claim 41 wherein said RecE and RecT or proteins are selected from E.coli RecE and RecT proteins or from phage λ Red α and Red β proteins.
- 30

43. A reagent kit for cloning comprising
(a) a host cell
(b) means of expressing recE and recT genes in said host cell and
(c) a recipient cloning vehicle capable of being replicated in said cell.
- 5
44. The reagent kit according to claim 43 wherein the means (b) comprise a vector system capable of expressing the recE and recT genes in the host cell.
- 10
45. The reagent kit according to claim 43 or 44 wherein the recE and recT genes are selected from E.coli recE and recT genes or from λ red α and red β genes.
- 15
46. A reagent kit for cloning comprising
(a) a source for RecE and RecT proteins and
(b) a recipient cloning vehicle capable of being propagated in a host cell.
- 20
47. The reagent kit according to claim 46 further comprising a host cell suitable for propagating said recipient cloning vehicle.
- 25
48. The reagent kit according to claim 46 or 47 wherein said RecE and RecT or proteins are selected from E.coli RecE and RecT proteins or from phage λ Red α and Red β proteins.
- 29
49. The reagent kit according to any one of claims 43-48 further comprising means for expressing a site specific recombinase in said host cell.
- 30
50. The reagent kit according to any one of claims 43-49 further comprising nucleic acid amplification primers comprising a region of homology to said recipient cloning vehicle.

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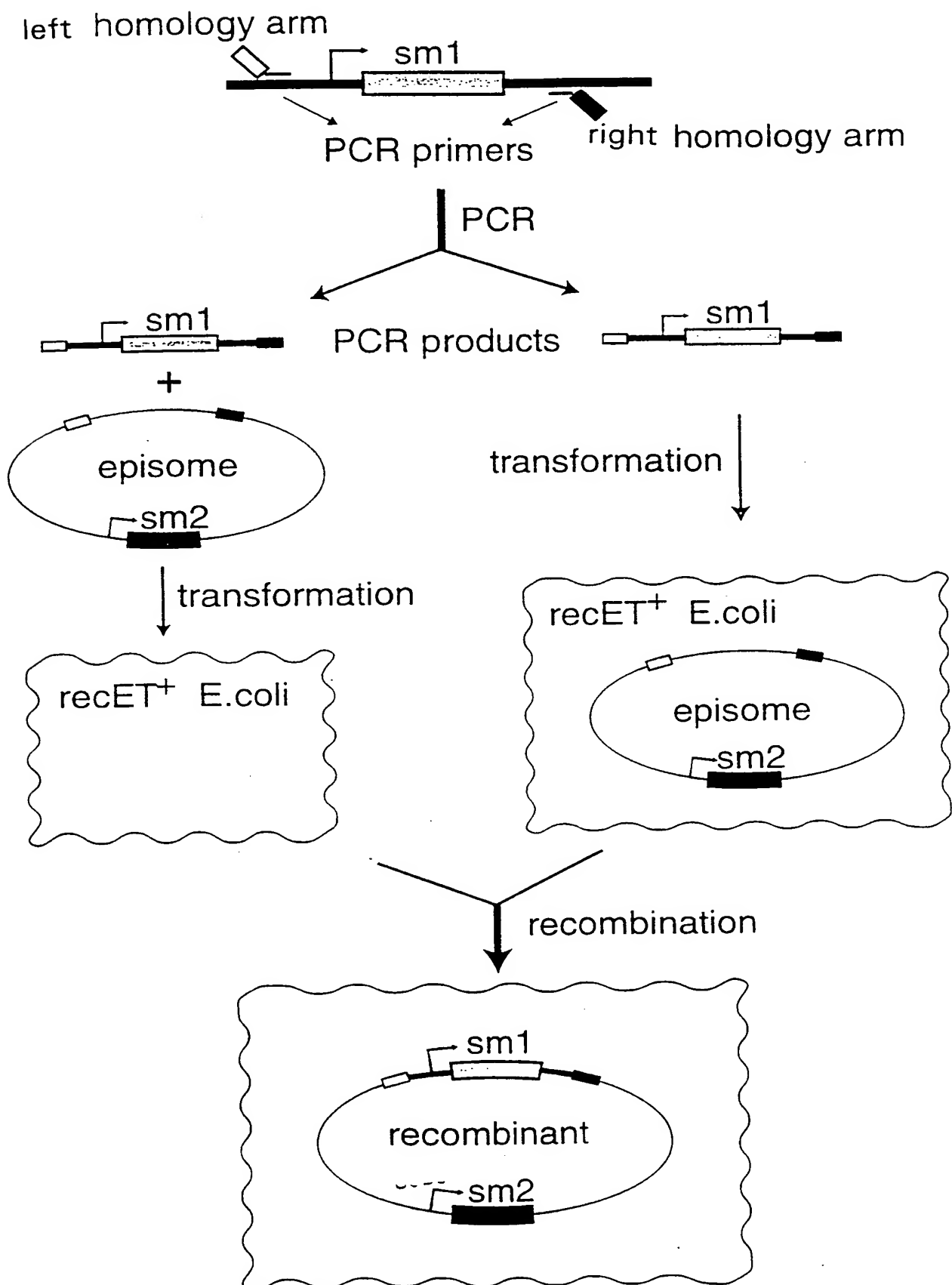


Figure 1

Three ways to select recombinants

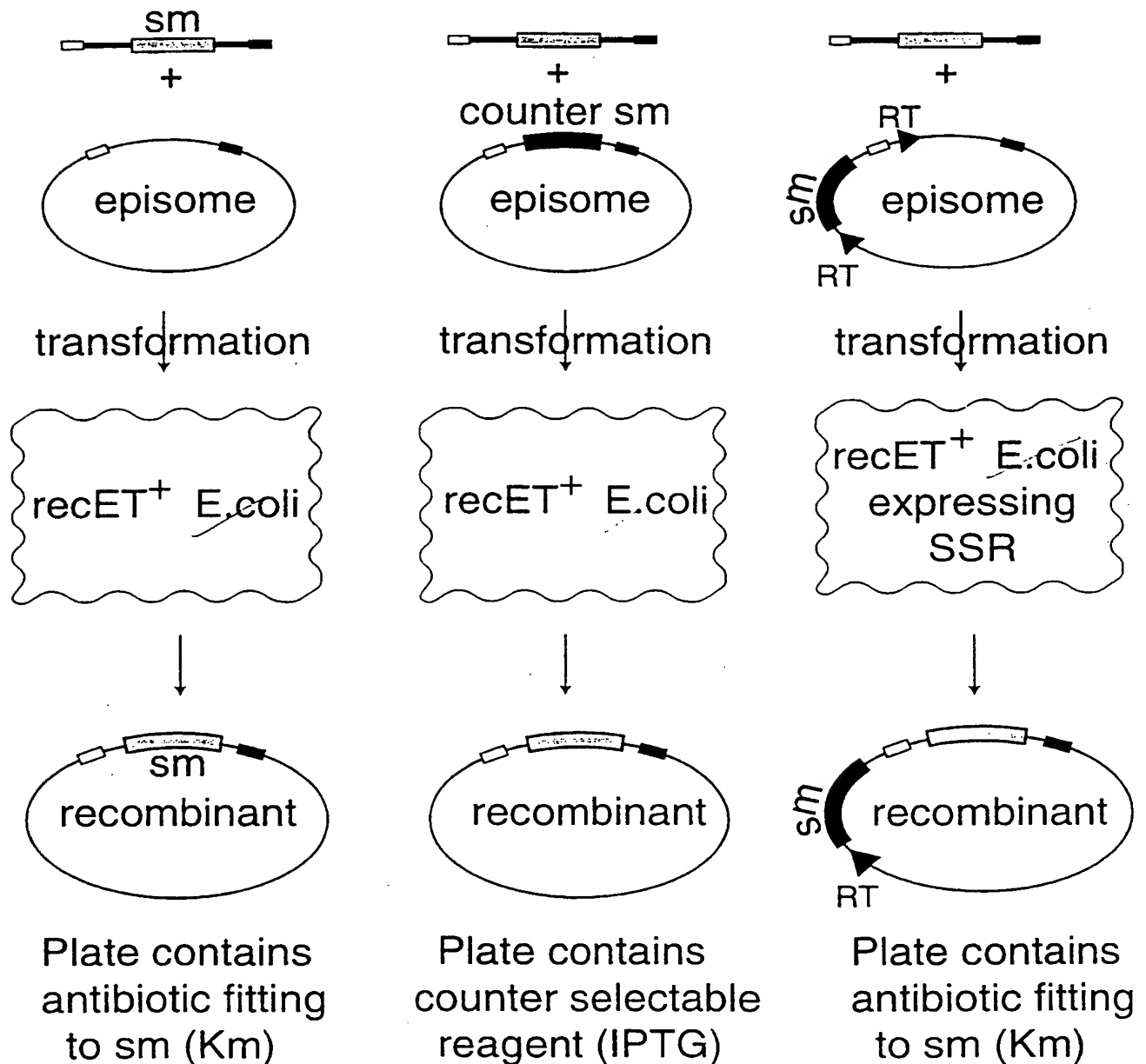
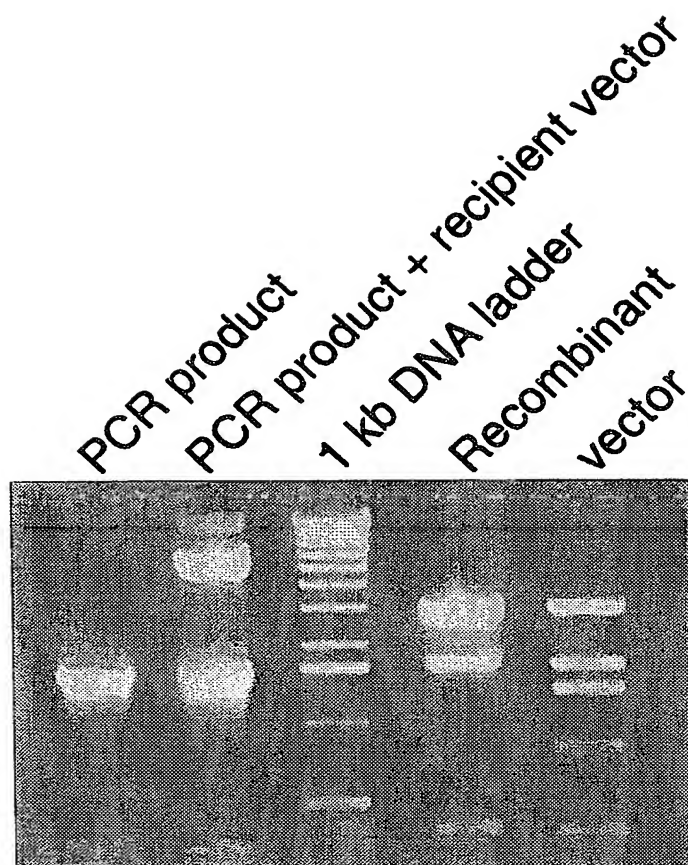
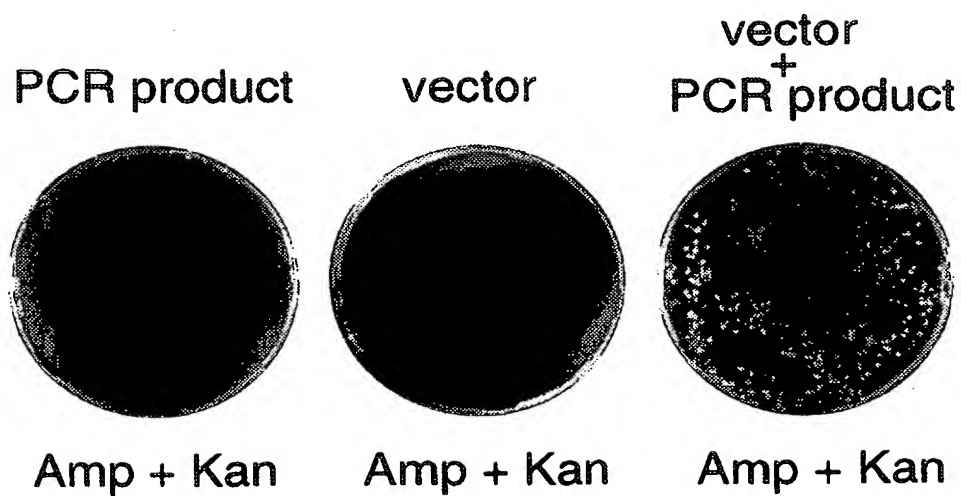


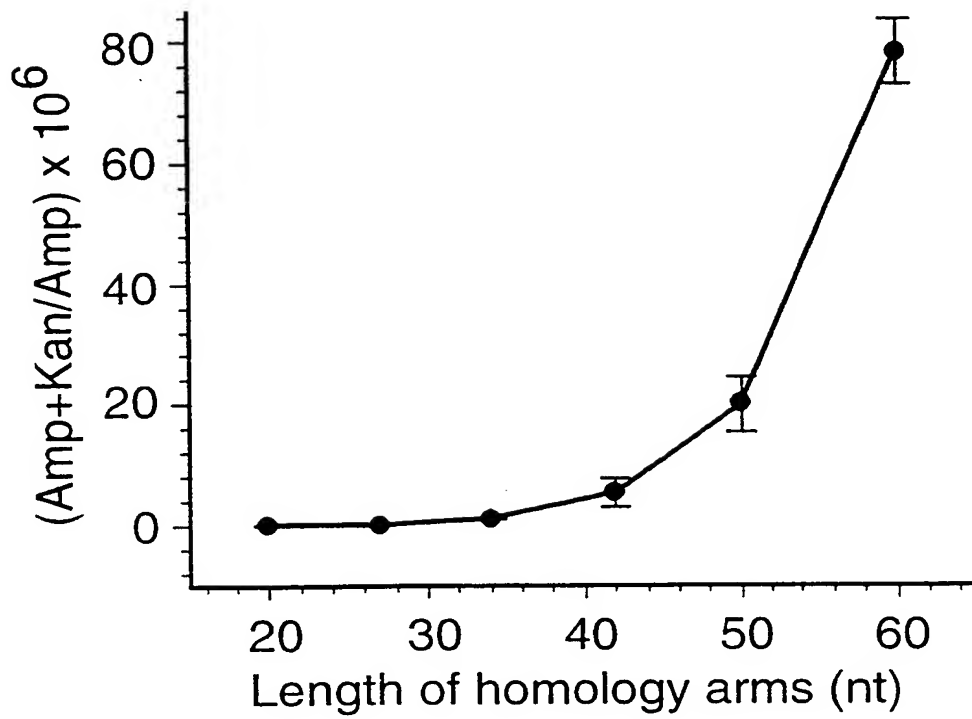
Figure 2

Figure 3

a*b*

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c



d

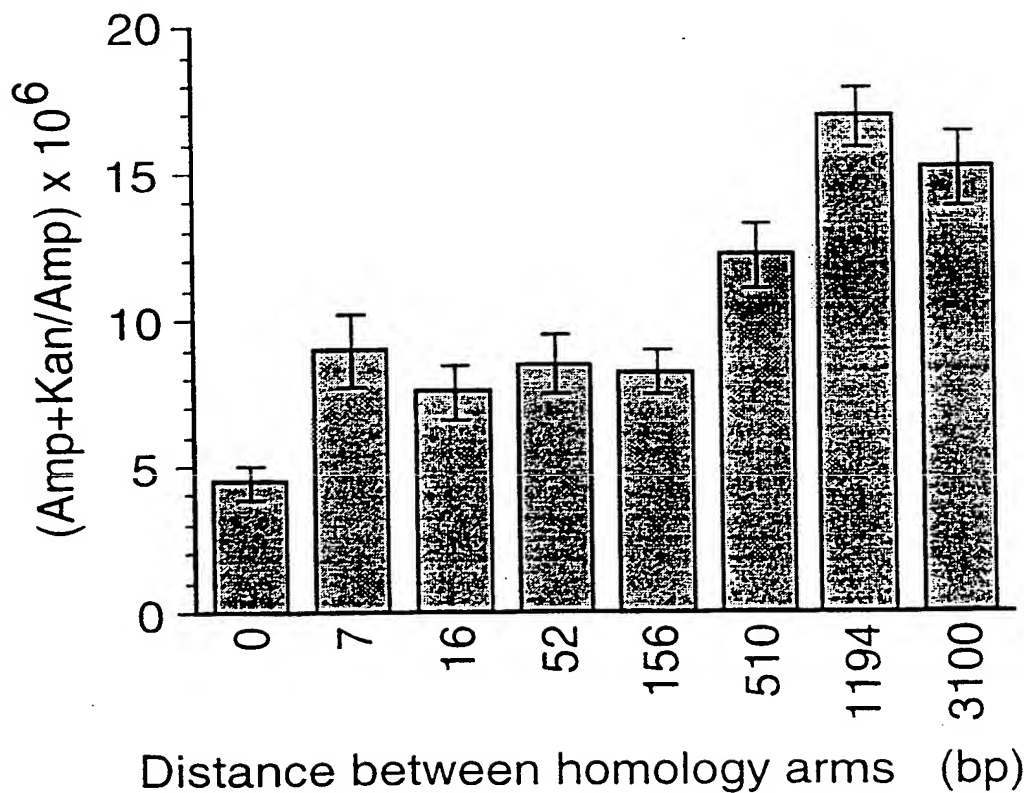


Figure 3

Figure 4a

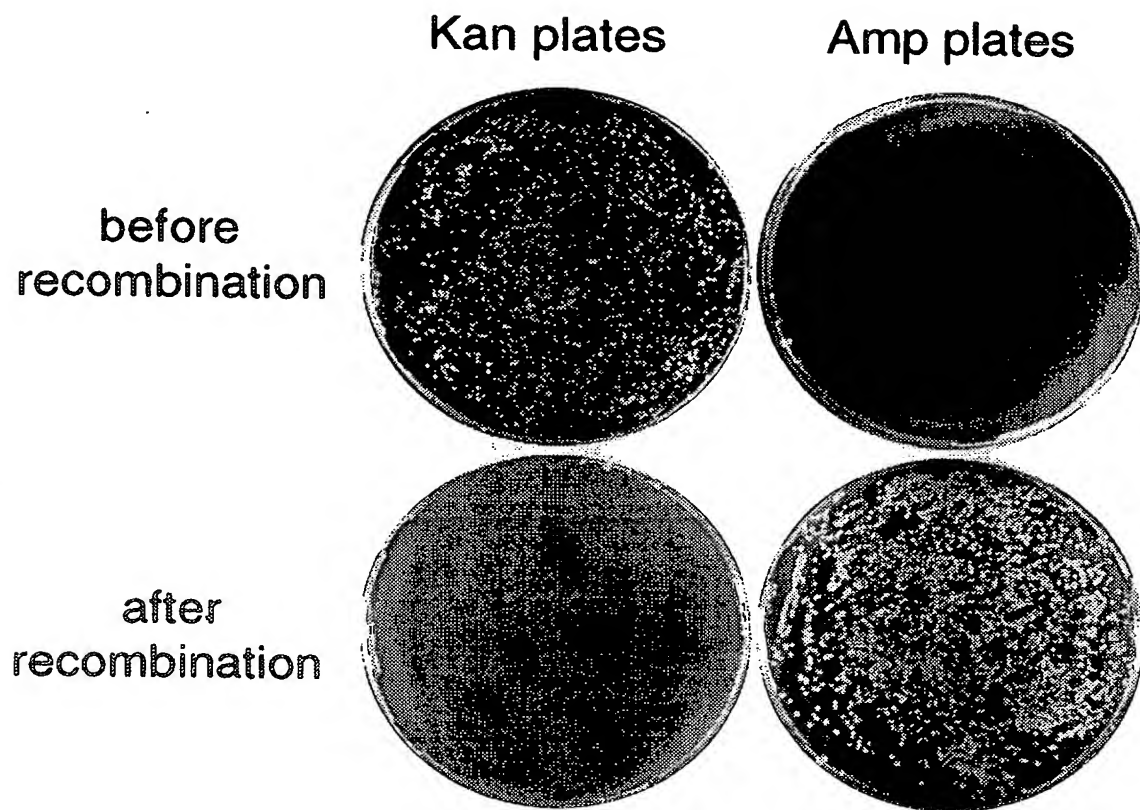
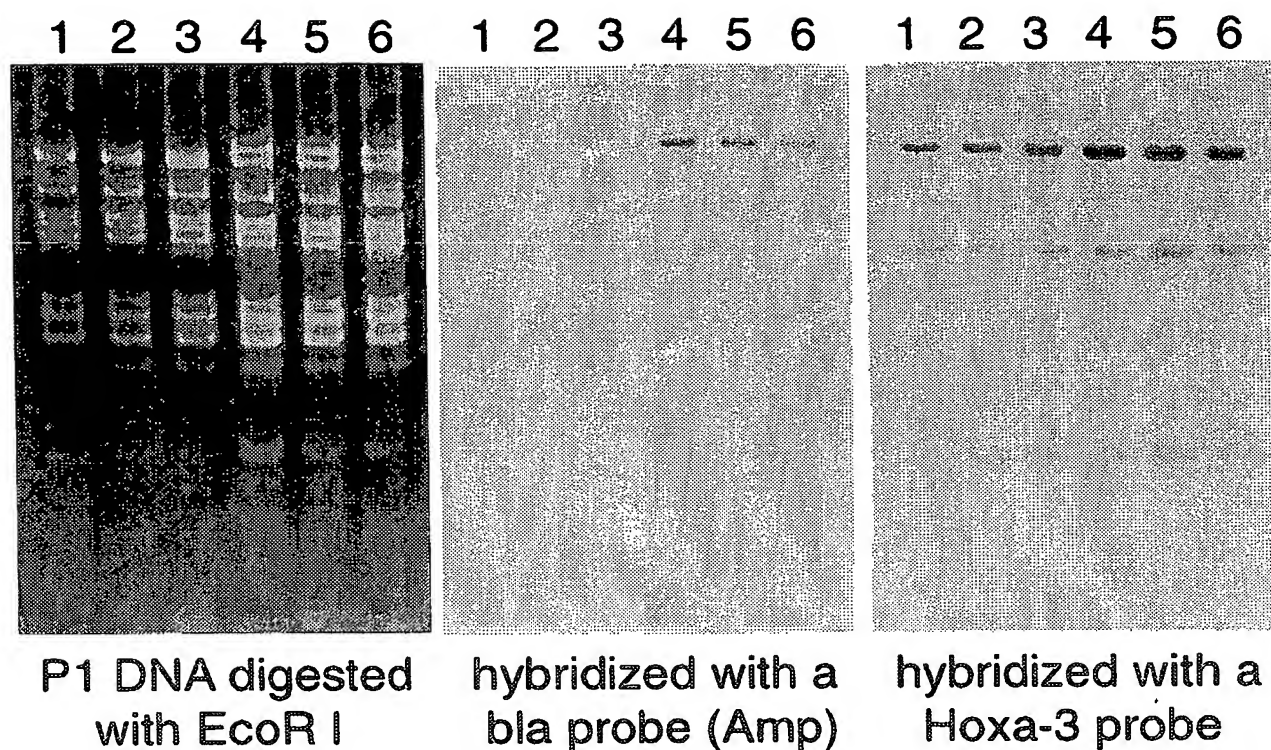


Figure 4b



- Lane 1: 1 of P1-Hox clone in NS3145 original bacterial strain (Kan resistance)
- Lane 2-3: 2 of P1-Hox clones in JC9604 before homologous recombination (Kan resistance)
- Lane 4-6: 3 of P1-Hox clones in JC9604 after homologous recombination (Amp resistance)

Figure 5

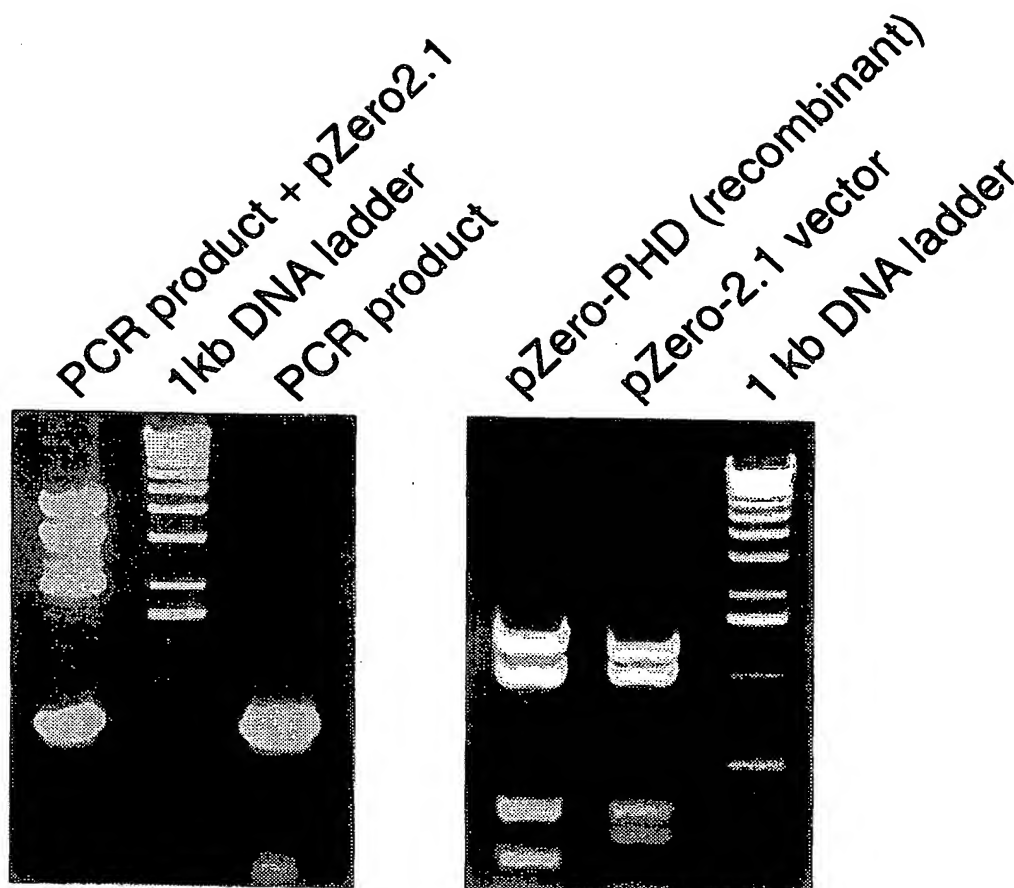
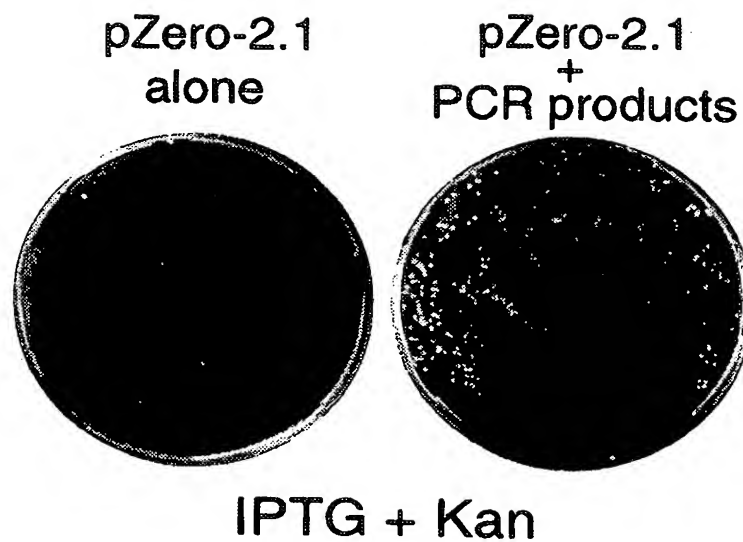
a*b*

Figure 6

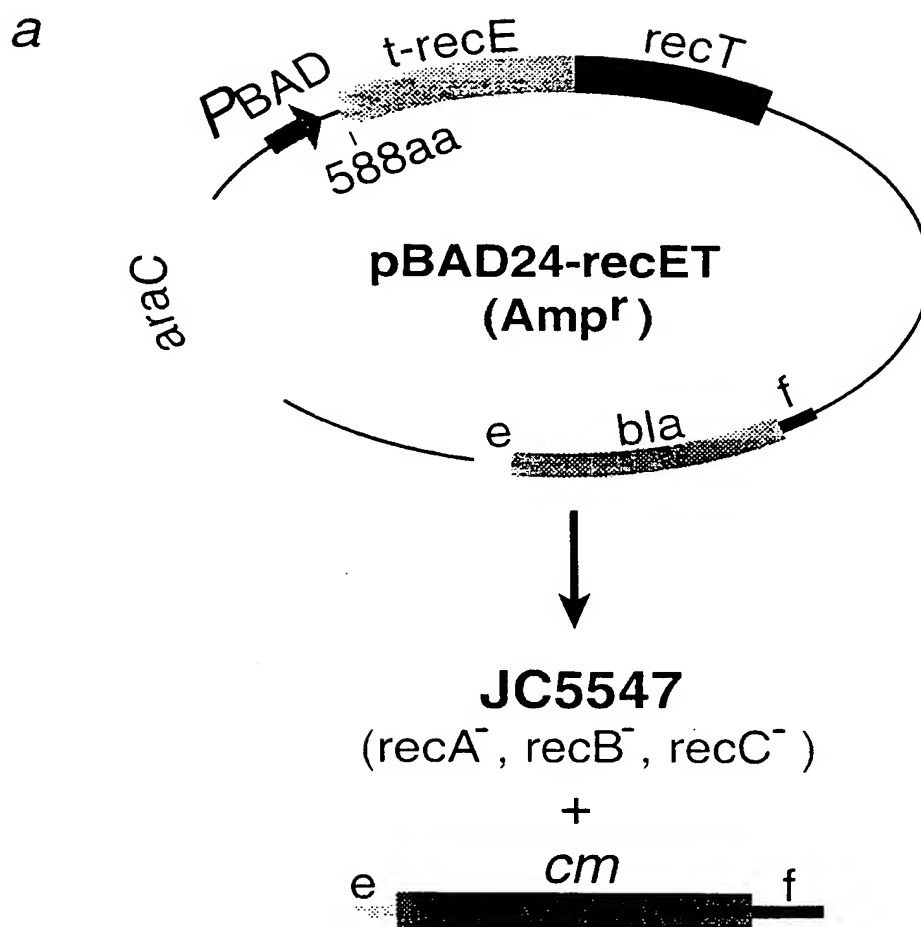
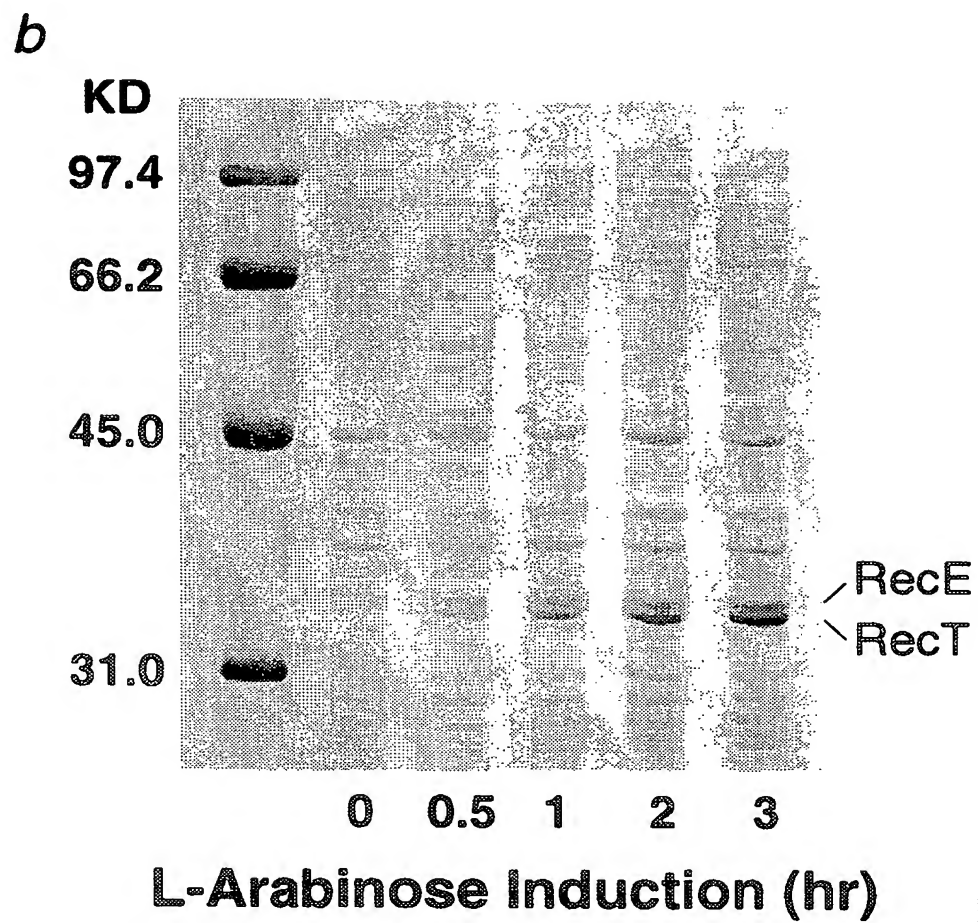


Figure 6



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C

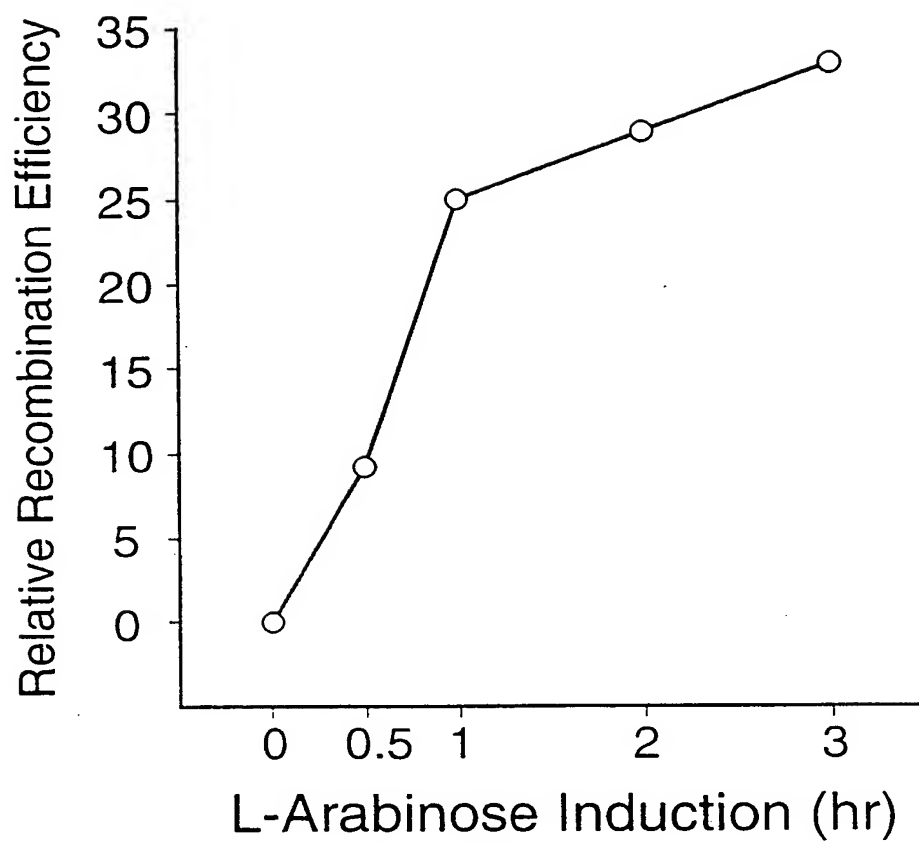
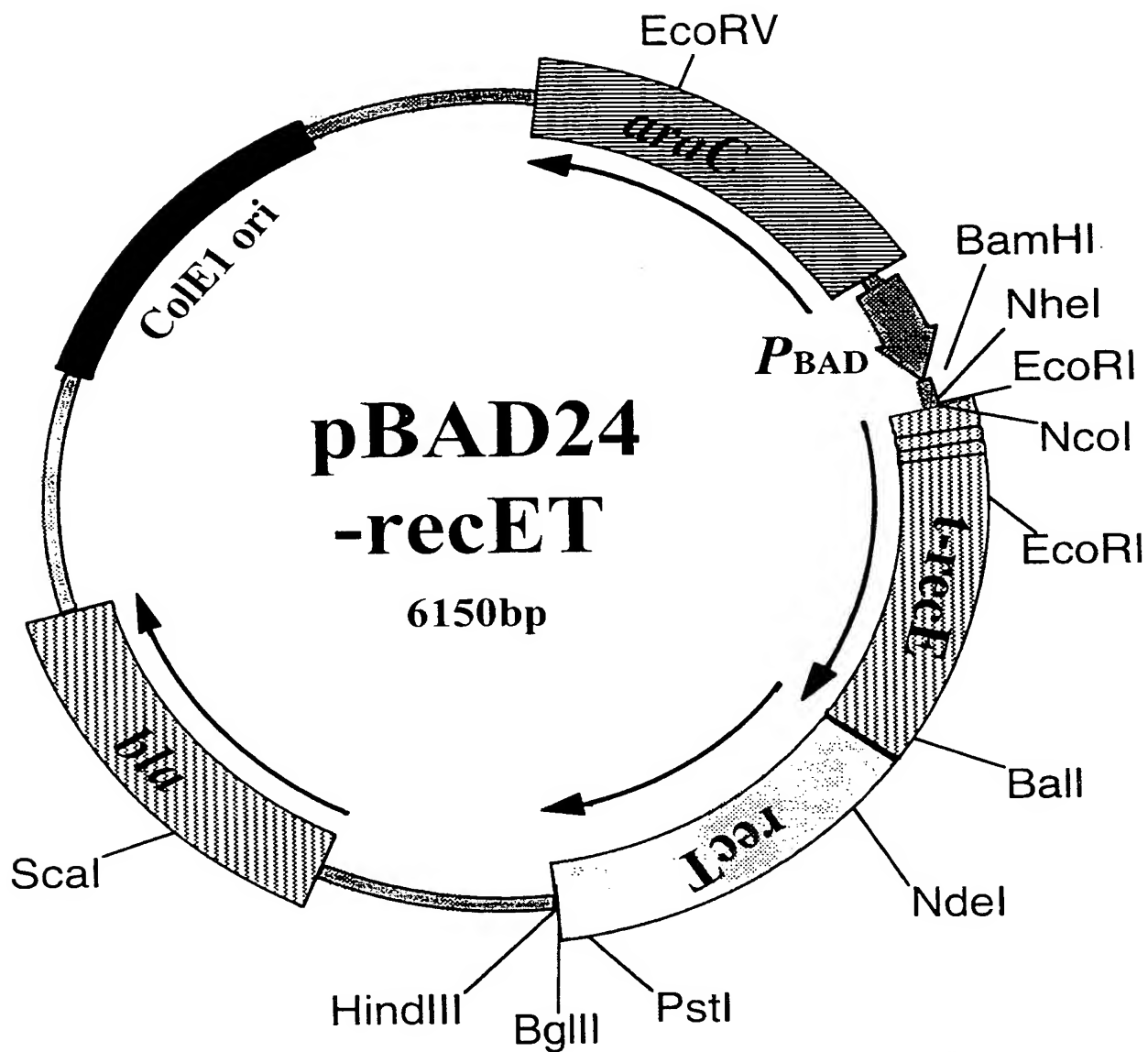


Figure 6

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Figure 7a



t-recE --- truncated recE (from 588 aa ---> end. 866 aa)

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Figure 7b

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1  ATCGATGCATAATGTGCCTGTCAAATGGACGAAGCAGGGATTTC
44  TGCAAACCCTATGCTACTCCGTCAAGCCGTCAATTGTCTGATT
87  CGTTACCAA TTA TGA CAA CTT GAC GGC TAC ATC
    293◀••• Ser Leu Lys Val Ala Val Asp

120  ATT CAC TTT TTC TTC ACA ACC GGC ACG GAA CTC
285◀Asn Val Lys Glu Glu Cys Gly Ala Arg Phe Glu

153  GCT CGG GCT GGC CCC GGT GCA TTT TTT AAA TAC
274◀Ser Pro Ser Ala Gly Thr Cys Lys Lys Phe Val

186  CCG CGA GAA ATA GAG TTG ATC GTC AAA ACC AAC
263◀Arg Ser Phe Tyr Leu Gln Asp Asp Phe Gly Val

219  ATT GCG ACC GAC GGT GGC GAT AGG CAT CCG GGT
252◀Asn Arg Gly Val Thr Ala Ile Pro Met Arg Thr

252  GGT GCT CAA AAG CAG CTT CGC CTG GCT GAT ACG
241◀Thr Ser Leu Leu Leu Lys Ala Gln Ser Ile Arg

285  TTG GTC CTC GCG CCA GCT TAA GAC GCT AAT CCC
230◀Gln Asp Glu Arg Trp Ser Leu Val Ser Ile Gly

318  TAA CTG CTG GCG GAA AAG ATG TGA CAG ACG CGA
219◀Leu Gln Gln Arg Phe Leu His Ser Leu Arg Ser

351  CGG CGA CAA GCA AAC ATG CTG TGC GAC GCT GGC
208◀Pro Ser Leu Cys Val His Gln Ala Val Ser Ala
    EcoRV

384  GAT ATC AAA ATT GCT GTC TGC CAG GTG ATC GCT
197◀Ile Asp Phe Asn Ser Asp Ala Leu His Asp Ser

417  GAT GTA CTG ACA AGC CTC GCG TAC CCG ATT ATC
186◀Ile Tyr Gln Cys Ala Glu Arg Val Arg Asn Asp

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Figure 7b (cont'd)

450	CAT	CGG	TGG	ATG	GAG	CGA	CTC	GTT	AAT	CGC	TTC
175	Met	Pro	Pro	His	Leu	Ser	Glu	Asn	Ile	Ala	Glu
483	CAT	GCG	CCG	CAG	TAA	CAA	TTG	CTC	AAG	CAG	ATT
164	Met	Arg	Arg	Leu	Leu	Leu	Gln	Glu	Leu	Leu	Asn
516	TAT	CGC	CAG	CAG	CTC	CGA	ATA	GCG	CCC	TTC	CCC
153	Ile	Ala	Leu	Leu	Glu	Ser	Tyr	Arg	Gly	Glu	Gly
549	TTG	CCC	GGC	GTT	AAT	GAT	TTG	CCC	AAA	CAG	GTC
142	Gln	Gly	Ala	Asn	Ile	Ile	Gln	Gly	Phe	Leu	Asp
582	GCT	GAA	ATG	CGG	CTG	GTG	CGC	TTC	ATC	CGG	GCG
131	Ser	Phe	His	Pro	Gln	His	Ala	Glu	Asp	Pro	Arg
615	AAA	GAA	CCC	CGT	ATT	GGC	AAA	TAT	TGA	CGG	CCA
120	Phe	Phe	Gly	Thr	Asn	Ala	Phe	Ile	Ser	Pro	Trp
648	GTT	AAG	CCA	TTC	ATG	CCA	GTA	GGC	GCG	CGG	ACG
109	Asn	Leu	Trp	Glu	His	Trp	Tyr	Ala	Arg	Pro	Arg
681	AAA	GTA	AAC	CCA	CTG	GTG	ATA	CCA	TTC	GCG	AGC
98	Phe	Tyr	Val	Trp	Gln	His	Tyr	Trp	Glu	Arg	Ala
714	CTC	CGG	ATG	ACG	ACC	GTA	GTG	ATG	AAT	CTC	TCC
87	Glu	Pro	His	Arg	Gly	Tyr	His	His	Ile	Glu	Gly
747	TGG	CGG	GAA	CAG	CAA	AAT	ATC	ACC	CGG	TCG	GCA
76	Pro	Pro	Phe	Leu	Leu	Ile	Asp	Gly	Pro	Arg	Cys
780	AAC	AAA	TTC	TCG	TCC	CTG	ATT	TTT	CAC	CAC	CCC
65	Val	Phe	Glu	Arg	Gly	Gln	Asn	Lys	Val	Val	Gly
813	CTG	ACC	GCG	AAT	GGT	GAG	ATT	GAG	AAT	ATA	ACC
54	Gln	Gly	Arg	Ile	Thr	Leu	Asn	Leu	Ile	Tyr	Gly
846	TTT	CAT	TCC	CAG	CGG	TCG	GTC	GAT	AAA	AAA	ATC
43	Lys	Met	Gly	Leu	Pro	Arg	Asp	Ile	Phe	Phe	Asp

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Figure 7b (cont'd)

879 GAG ATA ACC GTT GGC CTC AAT CGG CGT TAA ACC
 32◀ Leu Tyr Gly Asn Ala Glu Ile Pro Thr Leu Gly
 912 CGC CAC CAG ATG GGC ATT AAA CGA GTA TCC CGG
 21◀ Ala Val Leu His Ala Asn Phe Ser Tyr Gly Pro
 945 CAG CAG GGG ATC ATT TTG CGC TTC AGC CAT
 10◀ Leu Leu Pro Asp Asn Gln Ala Glu Ala Met
 975 ACTTTTCATA CTCCCGCCAT TCAGAGAAGA AACCAATTGT
 1015 CCATATTGCA TCAGACATTG CCGTCACTGC GTCTTTTACT
 1055 GGCTCTTCTC GCTAACCAAA CCGGTAACCC CGCTTATTAA
 1095 AAGCATTCTG TAACAAAGCG GGACCAAAGC CATGACAAAA
 1135 ACGCGTAACA AAAGTGTCTA TAATCACGGC AGAAAAGTCC
 1175 ACATTGATTA TTTGCACGGC GTCACACTTT GCTATGCCAT
 BamHI
 1215 AGCATTTTTA TCCATAAGAT TAGCGGATCC TACCTGACGC
 1255 TTTTATATCGC AACTCTCTAC TGTCTCTCCA TACCCGTTTT
 NheI EcoRI NcoI
 1295 TTTGGGCTAG CAGGAGGAAT TCACC ATG GAT CCC GTA
 1▶ Met Asp Pro Val
 1332 ATC GTA GAA GAC ATA GAG CCA GGT ATT TAT TAC
 5▶ Ile Val Glu Asp Ile Glu Pro Gly Ile Tyr Tyr
 1365 GGA ATT TCG AAT GAG AAT TAC CAC GCG GGT CCC
 16▶ Gly Ile Ser Asn Glu Asn Tyr His Ala Gly Pro
 1398 GGT ATC AGT AAG TCT CAG CTC GAT GAC ATT GCT

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Figure 7b (cont'd)

27▶ Gly Ile Ser Lys Ser Gln Leu Asp Asp Ile Ala
1431 GAT ACT CCG GCA CTA TAT TTG TGG CGT AAA AAT

38▶ Asp Thr Pro Ala Leu Tyr Leu Trp Arg Lys Asn
1464 GCC CCC GTG GAC ACC ACA AAG ACA AAA ACG CTC

49▶ Ala Pro Val Asp Thr Thr Lys Thr Lys Thr Leu
1497 GAT TTA GGA ACT GCT TTC CAC TGC CGG GTA CTT

60▶ Asp Leu Gly Thr Ala Phe His Cys Arg Val Leu
EcoRI
1530 GAA CCG GAA GAA TTC AGT AAC CGC TTT ATC GTA

71▶ Glu Pro Glu Glu Phe Ser Asn Arg Phe Ile Val
1563 GCA CCT GAA TTT AAC CGC CGT ACA AAC GCC GGA

82▶ Ala Pro Glu Phe Asn Arg Arg Thr Asn Ala Gly
1596 AAA GAA GAA GAG AAA GCG TTT CTG ATG GAA TGC

93▶ Lys Glu Glu Glu Lys Ala Phe Leu Met Glu Cys
1629 GCA AGC ACA GGA AAA ACG GTT ATC ACT GCG GAA

104▶ Ala Ser Thr Gly Lys Thr Val Ile Thr Ala Glu
1662 GAA GGC CGG AAA ATT GAA CTC ATG TAT CAA AGC

115▶ Glu Gly Arg Lys Ile Glu Leu Met Tyr Gln Ser

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Figure 7b (cont'd)

1695 GTT ATG GCT TTG CCG CTG GGG CAA TGG CTT GTT
126▶ Val Met Ala Leu Pro Leu Gly Gln Trp Leu Val
1728 GAA AGC GCC GGA CAC GCT GAA TCA TCA ATT TAC
137▶ Glu Ser Ala Gly His Ala Glu Ser Ser Ile Tyr
1761 TGG GAA GAT CCT GAA ACA GGA ATT TTG TGT CGG
148▶ Trp Glu Asp Pro Glu Thr Gly Ile Leu Cys Arg
1794 TGC CGT CCG GAC AAA ATT ATC CCT GAA TTT CAC
159▶ Cys Arg Pro Asp Lys Ile Ile Pro Glu Phe His
1827 TGG ATC ATG GAC GTG AAA ACT ACG GCG GAT ATT
170▶ Trp Ile Met Asp Val Lys Thr Thr Ala Asp Ile
1860 CAA CGA TTC AAA ACC GCT TAT TAC GAC TAC CGC
181▶ Gln Arg Phe Lys Thr Ala Tyr Tyr Asp Tyr Arg
1893 TAT CAC GTT CAG GAT GCA TTC TAC AGT GAC GGT
192▶ Tyr His Val Gln Asp Ala Phe Tyr Ser Asp Gly
1926 TAT GAA GCA CAG TTT GGA GTG CAG CCA ACT TTC
203▶ Tyr Glu Ala Gln Phe Gly Val Gln Pro Thr Phe
1959 GTT TTT CTG GTT GCC AGC ACA ACT ATT GAA TGC
214▶ Val Phe Leu Val Ala Ser Thr Thr Ile Glu Cys
1992 GGA CGT TAT CCG GTT GAA ATT TTC ATG ATG GGC

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Figure 7b (cont'd)

225▶ Gly Arg Tyr Pro Val Glu Ile Phe Met Met Gly
 2025 GAA GAA GCA AAA CTG GCA GGT CAA CAG GAA TAT

 236▶ Glu Glu Ala Lys Leu Ala Gly Gln Gln Glu Tyr
 2058 CAC CGC AAT CTG CGA ACC CTG TCT GAC TGC CTG

 247▶ His Arg Asn Leu Arg Thr Leu Ser Asp Cys Leu
 Ball
 2091 AAT ACC GAT GAA TGG CCA GCT ATT AAG ACA TTA

 258▶ Asn Thr Asp Glu Trp Pro Ala Ile Lys Thr Leu
 2124 TCA CTG CCC CGC TGG GCT AAG GAA TAT GCAA

 269▶ Ser Leu Pro Arg Trp Ala Lys Glu Tyr AlaA
 2155 ATG ACT AAG CAA CCA CCA ATC GCA AAA GCC GAT
 1▶ Met Thr Lys Gln Pro Pro Ile Ala Lys Ala Asp
 279▶ s nAs p•••

 2188 CTG CAA AAA ACT CAG GGA AAC CGT GCA CCA GCA
 12▶ Leu Gln Lys Thr Gln Gly Asn Arg Ala Pro Ala
 2221 GCA GTT AAA AAT AGC GAC GTG ATT AGT TTT ATT
 23▶ Ala Val Lys Asn Ser Asp Val Ile Ser Phe Ile
 2254 AAC CAG CCA TCA ATG AAA GAG CAA CTG GCA GCA
 34▶ Asn Gln Pro Ser Met Lys Glu Gln Leu Ala Ala
 Ndel
 2287 GCT CTT CCA CGC CAT ATG ACG GCT GAA CGT ATG
 45▶ Ala Leu Pro Arg His Met Thr Ala Glu Arg Met

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Figure 7b (cont'd)

2320	ATC	CGT	ATC	GCC	ACC	ACA	GAA	ATT	CGT	AAA	GTT
56▶	Ile	Arg	Ile	Ala	Thr	Thr	Glu	Ile	Arg	Lys	Val
2353	CCG	GCG	TTA	GGA	AAC	TGT	GAC	ACT	ATG	AGT	TTT
67▶	Pro	Ala	Leu	Gly	Asn	Cys	Asp	Thr	Met	Ser	Phe
2386	GTC	AGT	GCG	ATC	GTA	CAG	TGT	TCA	CAG	CTC	GGA
78▶	Val	Ser	Ala	Ile	Val	Gln	Cys	Ser	Gln	Leu	Gly
2419	CTT	GAG	CCA	GGT	AGC	GCC	CTC	GGT	CAT	GCA	TAT
89▶	Leu	Glu	Pro	Gly	Ser	Ala	Leu	Gly	His	Ala	Tyr
2452	TTA	CTG	CCT	TTT	GGT	AAT	AAA	AAC	GAA	AAG	AGC
100▶	Leu	Leu	Pro	Phe	Gly	Asn	Lys	Asn	Glu	Lys	Ser
2485	GGT	AAA	AAG	AAC	GTT	CAG	CTA	ATC	ATT	GGC	TAT
111▶	Gly	Lys	Lys	Asn	Val	Gln	Leu	Ile	Ile	Gly	Tyr
2518	CGC	GGC	ATG	ATT	GAT	CTG	GCT	CGC	CGT	TCT	GGT
122▶	Arg	Gly	Met	Ile	Asp	Leu	Ala	Arg	Arg	Ser	Gly
2551	CAA	ATC	GCC	AGC	CTG	TCA	GCC	CGT	GTT	GTC	CGT
133▶	Gln	Ile	Ala	Ser	Leu	Ser	Ala	Arg	Val	Val	Arg
2584	GAA	GGT	GAC	GAG	TTT	AGC	TTC	GAA	TTT	GGC	CTT
144▶	Glu	Gly	Asp	Glu	Phe	Ser	Phe	Glu	Phe	Gly	Leu
2617	GAT	GAA	AAG	TTA	ATA	CAC	CGC	CCG	GGA	GAA	AAC
155▶	Asp	Glu	Lys	Leu	Ile	His	Arg	Pro	Gly	Glu	Asn
2650	GAA	GAT	GCC	CCG	GTT	ACC	CAC	GTC	TAT	GCT	GTC
166▶	Glu	Asp	Ala	Pro	Val	Thr	His	Val	Tyr	Ala	Val
2683	GCA	AGA	CTG	AAA	GAC	GGA	GGT	ACT	CAG	TTT	GAA
177▶	Ala	Arg	Leu	Lys	Asp	Gly	Gly	Thr	Gln	Phe	Glu
2716	GTT	ATG	ACG	CGC	AAA	CAG	ATT	GAG	CTG	GTG	CGC
188▶	Val	Met	Thr	Arg	Lys	Gln	Ile	Glu	Leu	Val	Arg

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Figure 7b (cont'd)

2749 AGC CTG AGT AAA GCT GGT AAT AAC GGG CCG TGG
 199► Ser Leu Ser Lys Ala Gly Asn Asn Gly Pro Trp
 2782 GTA ACT CAC TGG GAA GAA ATG GCA AAG AAA ACG
 210► Val Thr His Trp Glu Glu Met Ala Lys Lys Thr
 2815 GCT ATT CGT CGC CTG TTC AAA TAT TTG CCC GTA
 221► Ala Ile Arg Arg Leu Phe Lys Tyr Leu Pro Val
 2848 TCA ATT GAG ATC CAG CGT GCA GTA TCA ATG GAT
 232► Ser Ile Glu Ile Gln Arg Ala Val Ser Met Asp
 PstI
 2881 GAA AAG GAA CCA CTG ACA ATC GAT CCT GCA GAT
 243► Glu Lys Glu Pro Leu Thr Ile Asp Pro Ala Asp
 2914 TCC TCT GTA TTA ACC GGG GAA TAC AGT GTA ATC
 254► Ser Ser Val Leu Thr Gly Glu Tyr Ser Val Ile
 BglII HindIII
 2947 GAT AAT TCA GAG GAA TAG ATCTAAGCTT
 265► Asp Asn Ser Glu Glu • • •
 2975 GGCTGTTTTG GCGGATGAGA GAAGATTTTC AGCCTGATAC
 3015 AGATTAAATC AGAACGCAGA AGCGGTCTGA TAAACAGAA
 3055 TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC
 3095 ATGCCGAAC T CAGAAGTGAA ACGCCGTAGC GCCGATGGTA
 3135 GTGTGGGGTC TCCCCATGCG AGAGTAGGGA ACTGCCAGGC
 3175 ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT
 3215 TCGTTTTATC TGTGTGTTTGT CGGTGAACGC TCTCCTGAGT
 3255 AGGACAAATC CGCCGGGAGC GGATTTGAAC GTTGCGAAGC
 3295 AACGGCCCGG AGGGTGGCGG GCAGGACGCC CGCCATAAAC
 3335 TGCCAGGCAT CAAATTAAGC AGAAGGCCAT CCTGACGGAT

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Figure 7b (cont'd)

3375 GGCCTTTTTCG CGTTTCTACA AACTCTTTTCG TTTATTTTTC
 3415 TAAATACATT CAAATATGTA TCCGCTCATG AGACAATAAC
 3455 CCTGATAAAT GCTTCAATAA TATTGAAAAA GGAAGAGT AT
 1► Me
 3495 G AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT
 1► t Ser Ile Gln His Phe Arg Val Ala Leu Ile
 3526 CCC TTT TTT GCG GCA TTT TGC CTT CCT GTT TTT
 12► Pro Phe Phe Ala Ala Phe Cys Leu Pro Val Phe
 3559 GCT CAC CCA GAA ACG CTG GTG AAA GTA AAA GAT
 23► Ala His Pro Glu Thr Leu Val Lys Val Lys Asp
 3592 GCT GAA GAT CAG TTG GGT GCA CGA GTG GGT TAC
 34► Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr
 3625 ATC GAA CTG GAT CTC AAC AGC GGT AAG ATC CTT
 45► Ile Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu
 3658 GAG AGT TTT CGC CCC GAA GAA CGT TTT CCA ATG
 56► Glu Ser Phe Arg Pro Glu Glu Arg Phe Pro Met
 3691 ATG AGC ACT TTT AAA GTT CTG CTA TGT GGC GCG
 67► Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala
 3724 GTA TTA TCC CGT GTT GAC GCC GGG CAA GAG CAA
 78► Val Leu Ser Arg Val Asp Ala Gly Gln Glu Gln
 3757 CTC GGT CGC CGC ATA CAC TAT TCT CAG AAT GAC
 89► Leu Gly Arg Arg Ile His Tyr Ser Gln Asn Asp
 Scal
 3790 TTG GTT GAG TAC TCA CCA GTC ACA GAA AAG CAT
 100► Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His
 3823 CTT ACG GAT GGC ATG ACA GTA AGA GAA TTA TGC
 111► Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys

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Figure 7b (cont'd)

3856	AGT	GCT	GCC	ATA	ACC	ATG	AGT	GAT	AAC	ACT	GCG
122▶	Ser	Ala	Ala	Ile	Thr	Met	Ser	Asp	Asn	Thr	Ala
3889	GCC	AAC	TTA	CTT	CTG	ACA	ACG	ATC	GGA	GGA	CCG
133▶	Ala	Asn	Leu	Leu	Leu	Thr	Thr	Ile	Gly	Gly	Pro
3922	AAG	GAG	CTA	ACC	GCT	TTT	TTG	CAC	AAC	ATG	GGG
144▶	Lys	Glu	Leu	Thr	Ala	Phe	Leu	His	Asn	Met	Gly
3955	GAT	CAT	GTA	ACT	CGC	CTT	GAT	CGT	TGG	GAA	CCG
155▶	Asp	His	Val	Thr	Arg	Leu	Asp	Arg	Trp	Glu	Pro
3988	GAG	CTG	AAT	GAA	GCC	ATA	CCA	AAC	GAC	GAG	CGT
166▶	Glu	Leu	Asn	Glu	Ala	Ile	Pro	Asn	Asp	Glu	Arg
4021	GAC	ACC	ACG	ATG	CCT	GTA	GCA	ATG	GCA	ACA	ACG
177▶	Asp	Thr	Thr	Met	Pro	Val	Ala	Met	Ala	Thr	Thr
4054	TTG	CGC	AAA	CTA	TTA	ACT	GGC	GAA	CTA	CTT	ACT
188▶	Leu	Arg	Lys	Leu	Leu	Thr	Gly	Glu	Leu	Leu	Thr
4087	CTA	GCT	TCC	CGG	CAA	CAA	TTA	ATA	GAC	TGG	ATG
199▶	Leu	Ala	Ser	Arg	Gln	Gln	Leu	Ile	Asp	Trp	Met
4120	GAG	GCG	GAT	AAA	GTT	GCA	GGA	CCA	CTT	CTG	CGC
210▶	Glu	Ala	Asp	Lys	Val	Ala	Gly	Pro	Leu	Leu	Arg
4153	TCG	GCC	CTT	CCG	GCT	GGC	TGG	TTT	ATT	GCT	GAT
221▶	Ser	Ala	Leu	Pro	Ala	Gly	Trp	Phe	Ile	Ala	Asp
4186	AAA	TCT	GGA	GCC	GGT	GAG	CGT	GGG	TCT	CGC	GGT
232▶	Lys	Ser	Gly	Ala	Gly	Glu	Arg	Gly	Ser	Arg	Gly
4219	ATC	ATT	GCA	GCA	CTG	GGG	CCA	GAT	GGT	AAG	CCC
243▶	Ile	Ile	Ala	Ala	Leu	Gly	Pro	Asp	Gly	Lys	Pro
4252	TCC	CGT	ATC	GTA	GTT	ATC	TAC	ACG	ACG	GGG	AGT
254▶	Ser	Arg	Ile	Val	Val	Ile	Tyr	Thr	Thr	Gly	Ser

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Figure 7b (cont'd)

4285 CAG GCA ACT ATG GAT GAA CGA AAT AGA CAG ATC
265► Gln Ala Thr Met Asp Glu Arg Asn Arg Gln Ile

4318 GCT GAG ATA GGT GCC TCA CTG ATT AAG CAT TGG
276► Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp

4351 TAA CTGTCAGACC AAGTTTACTC ATATATACTT
287► . . .

4384 TAGATTGATT TACGCGCCCT GTAGCGGCGC ATTAAGCGCG
4424 GCGGGTGTGG TGGTTACGCG CAGCGTGACC GCTACACTTG
4464 CCAGCGCCCT AGCGCCCGCT CCTTTCGCTT TCTTCCCTTC
4504 CTTTCTCGCC ACGTTCGCCG GCTTTCCTCCG TCAAGCTCTA
4544 AATCGGGGGC TCCCTTTAGG GTTCCGATTT AGTGCTTTAC
4584 GGCACCTCGA CCCCAAAAAA CTTGATTTGG GTGATGGTTC
4624 ACGTAGTGGG CCATCGCCCT GATAGACGGT TTTTCGCCCT
4664 TTGACGTTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT
4704 TCCAAACTTG AACAACTC AACCTATCT CGGGCTATTC
4744 TTTTGATTTA TAAGGGATTT TGCCGATTTT GGCCTATTGG
4784 TTAAAAAATG AGCTGATTTA ACAAAAATTT AACGCGAATT
4824 TTAACAAAAT ATTAACGTTT ACAATTTAAA AGGATCTAGG
4864 TGAAGATCCT TTTTGATAAT CTCATGACCA AAATCCCTTA
4904 ACGTGAGTTT TCGTTCCACT GAGCGTCAGA CCCCCTAGAA
4944 AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG
4984 TAATCTGCTG CTTGCAAACA AAAAAACCAC CGCTACCAGC
5024 GGTGGTTTGT TTGCCGGATC AAGAGCTACC AACTCTTTTT

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Figure 7b (cont'd)

5064	CCGAAGGTAA	CTGGCTTCAG	CAGAGCGCAG	ATACCAAATA
5104	CTGTCCTTCT	AGTGTAGCCG	TAGTTAGGCC	ACCACTTCAA
5144	GAACTCTGTA	GCACCGCCTA	CATACCTCGC	TCTGCTAATC
5184	CTGTTACCAG	TGGCTGCTGC	CAGTGGCGAT	AAGTCGTGTC
5224	TTACCGGGTT	GGACTCAAGA	CGATAGTTAC	CGGATAAGGC
5264	GCAGCGGTCTG	GGCTGAACGG	GGGGTTCGTG	CACACAGCCC
5304	AGCTTGGAGC	GAACGACCTA	CACCGAACTG	AGATACCTAC
5344	AGCGTGAGCT	ATGAGAAAGC	GCCACGCTTC	CCGAAGGGAG
5384	AAAGGCGGAC	AGGTATCCGG	TAAGCGGCAG	GGTCGGAACA
5424	GGAGAGCGCA	CGAGGGAGCT	TCCAGGGGGA	AACGCCTGGT
5464	ATCTTTATAG	TCCTGTCGGG	TTTCGCCACC	TCTGACTTGA
5504	GCGTCGATTT	TTGTGATGCT	CGTCAGGGGG	GCGGAGCCTA
5544	TGGAAAAACG	CCAGCAACGC	GGCCTTTTTA	CGGTTTCCTGG
5584	CCTTTTGCTG	GCCTTTTGCT	CACATGTTCT	TTCTGCGTTT
5624	ATCCCCTGAT	TCTGTGGATA	ACCGTATTAC	CGCCTTTGAG
5664	TGAGCTGATA	CCGCTCGCCG	CAGCCGAACG	ACCGAGCGCA
5704	GCGAGTCAGT	GAGCGAGGAA	GCGGAAGAGC	GCCTGATGCG
5744	GTATTTTCTC	CTTACGCATC	TGTGCGGTAT	TTACACCCGC
5784	ATAGGGTCAT	GGCTGCGCCC	CGACACCCGC	CAACACCCGC
5824	TGACGCGCCC	TGACGGGCTT	GTCTGCTCCC	GGCATCCGCT
5864	TACAGACAAG	CTGTGACCGT	CTCCGGGAGC	TGCATGTGTC
5904	AGAGGTTTTTC	ACCGTCATCA	CCGAAACGCG	CGAGGCAGCA
5944	AGGAGATGGC	GCCCAACAGT	CCCCCGGCCA	CGGGGCCTGC

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Figure 7b (cont'd)

5984 CACCATACCC ACGCCGAAAC AAGCGCTCAT GAGCCCGAAG
6024 TGGCGAGCCC GATCTTCCCC ATCGGTGATG TCGGCGATAT
6064 AGGCGCCAGC AACCGCACCT GTGGCGCCGG TGATGCCGGC
6104 CACGATGCGT CCGGCGTAGA GGATCTGCTC ATGTTTGACA
6144 GCTTATC

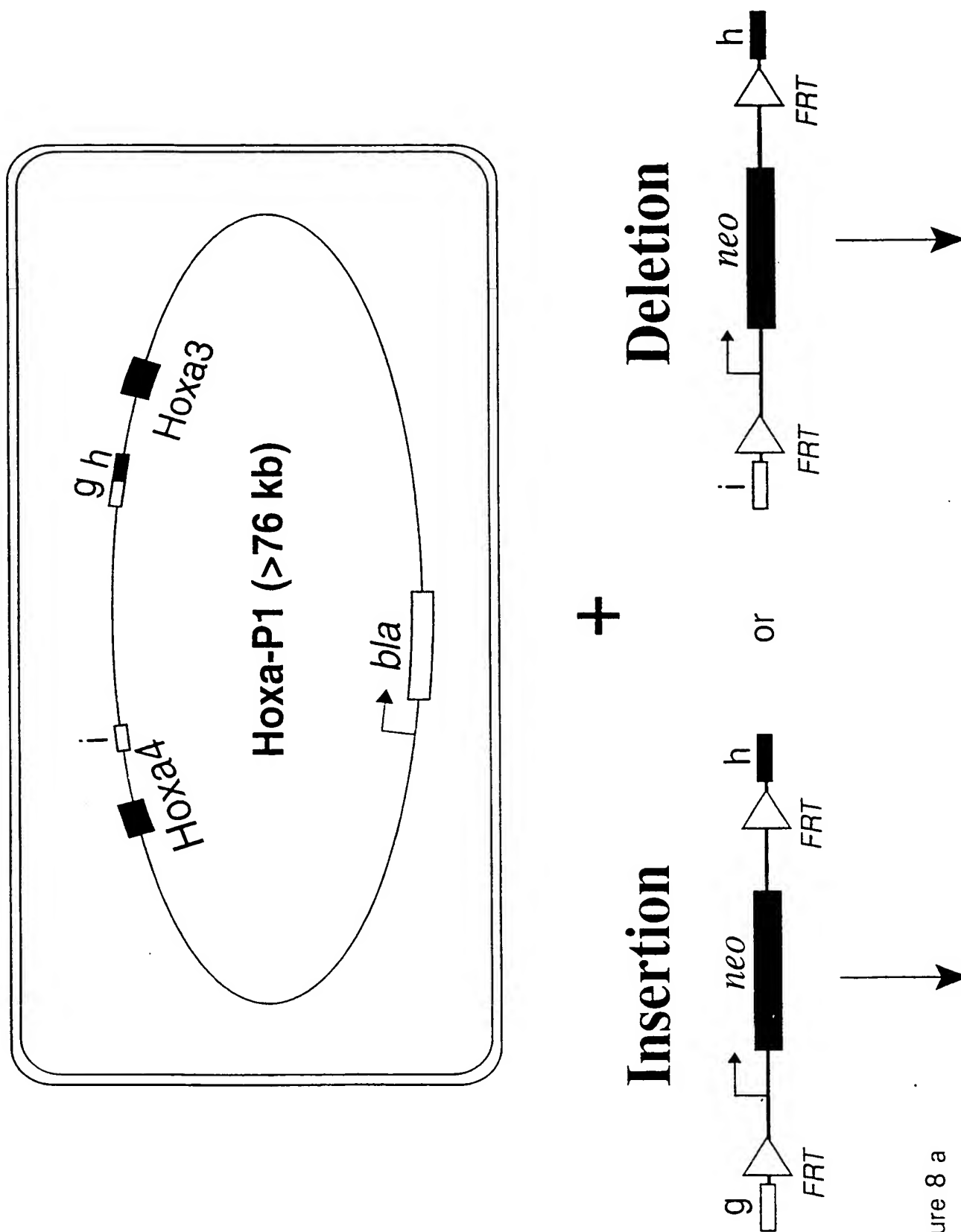


Figure 8 a

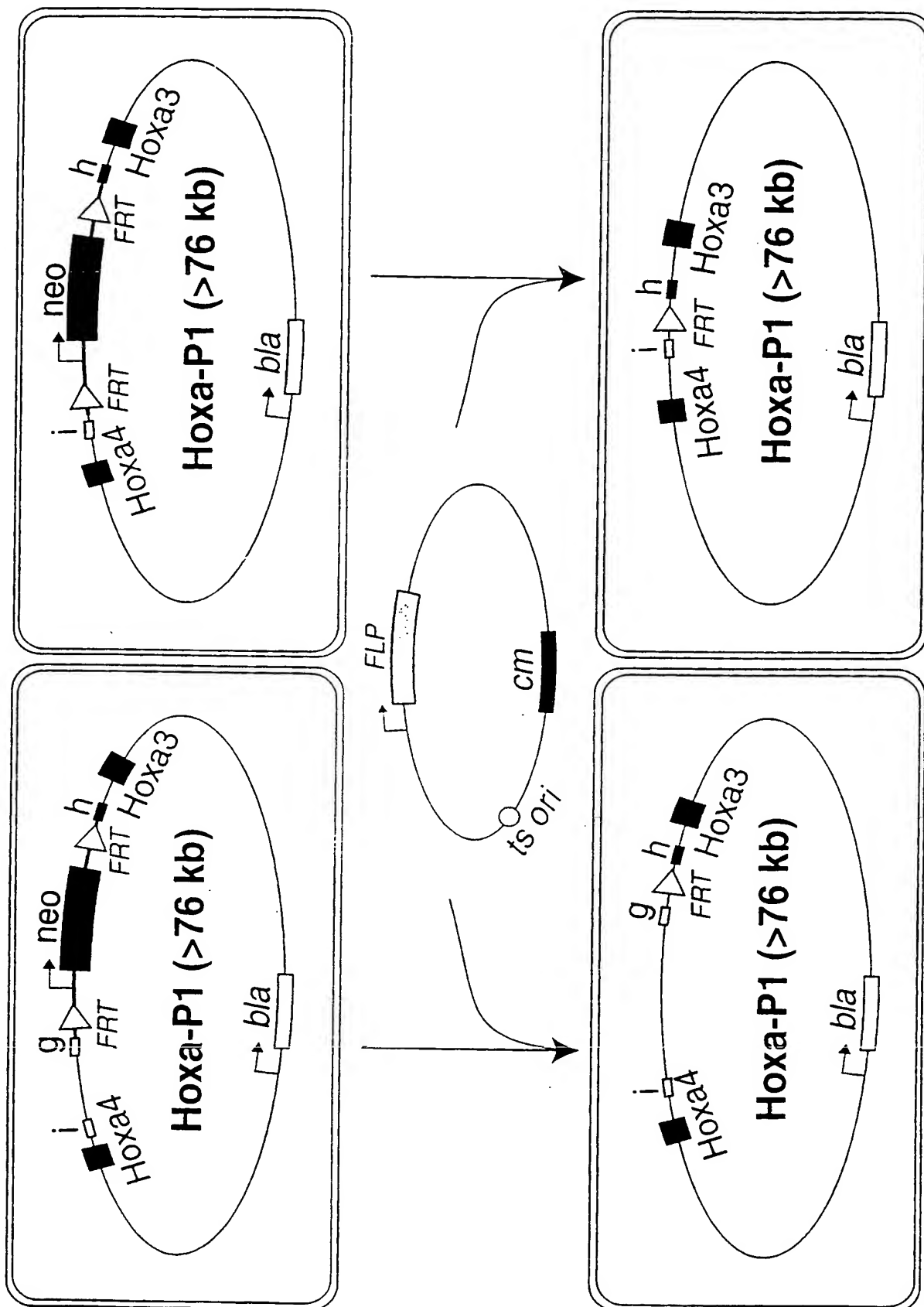


Figure 8 a (continuing)

Figure 8 b

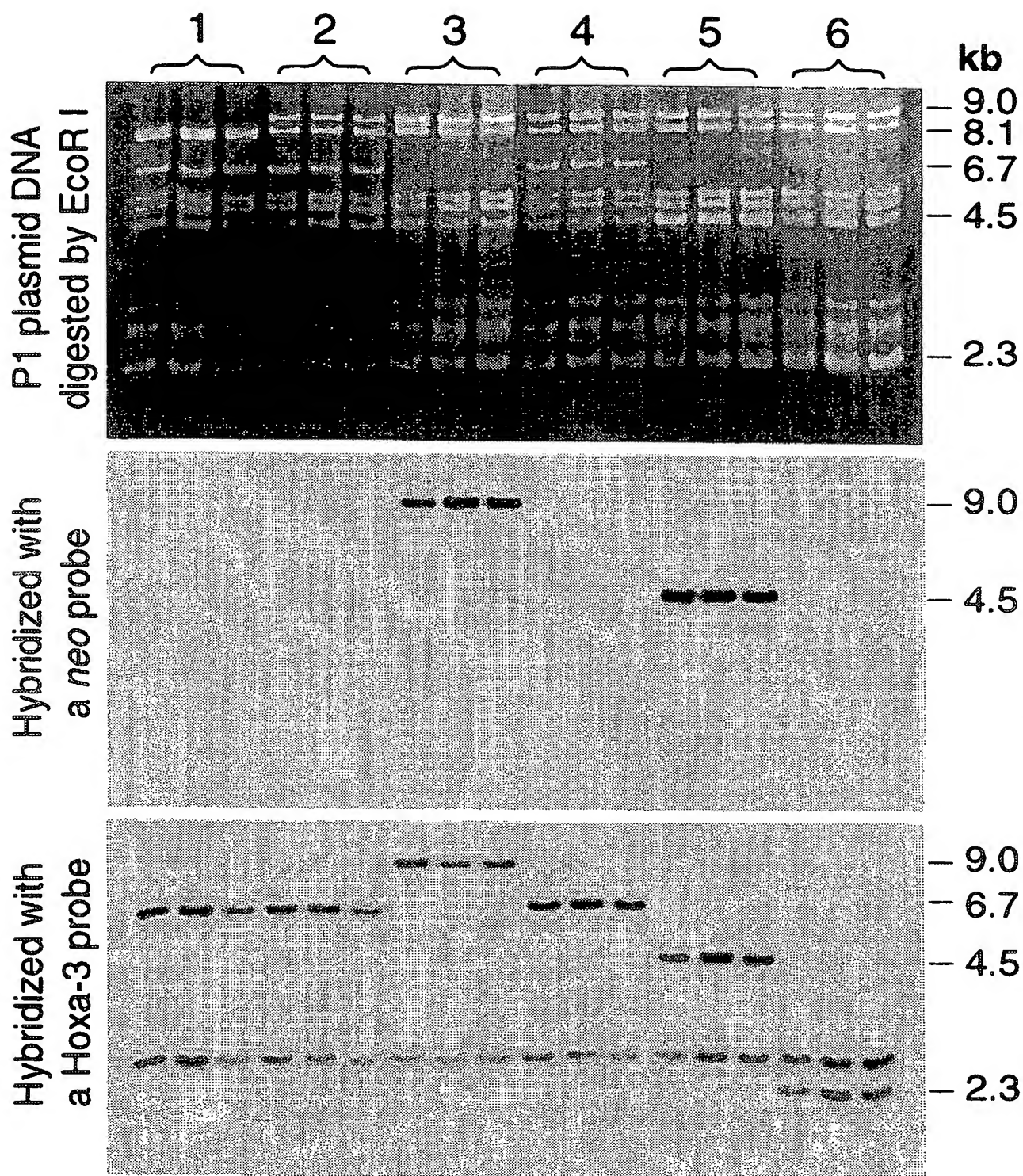


Figure 9a

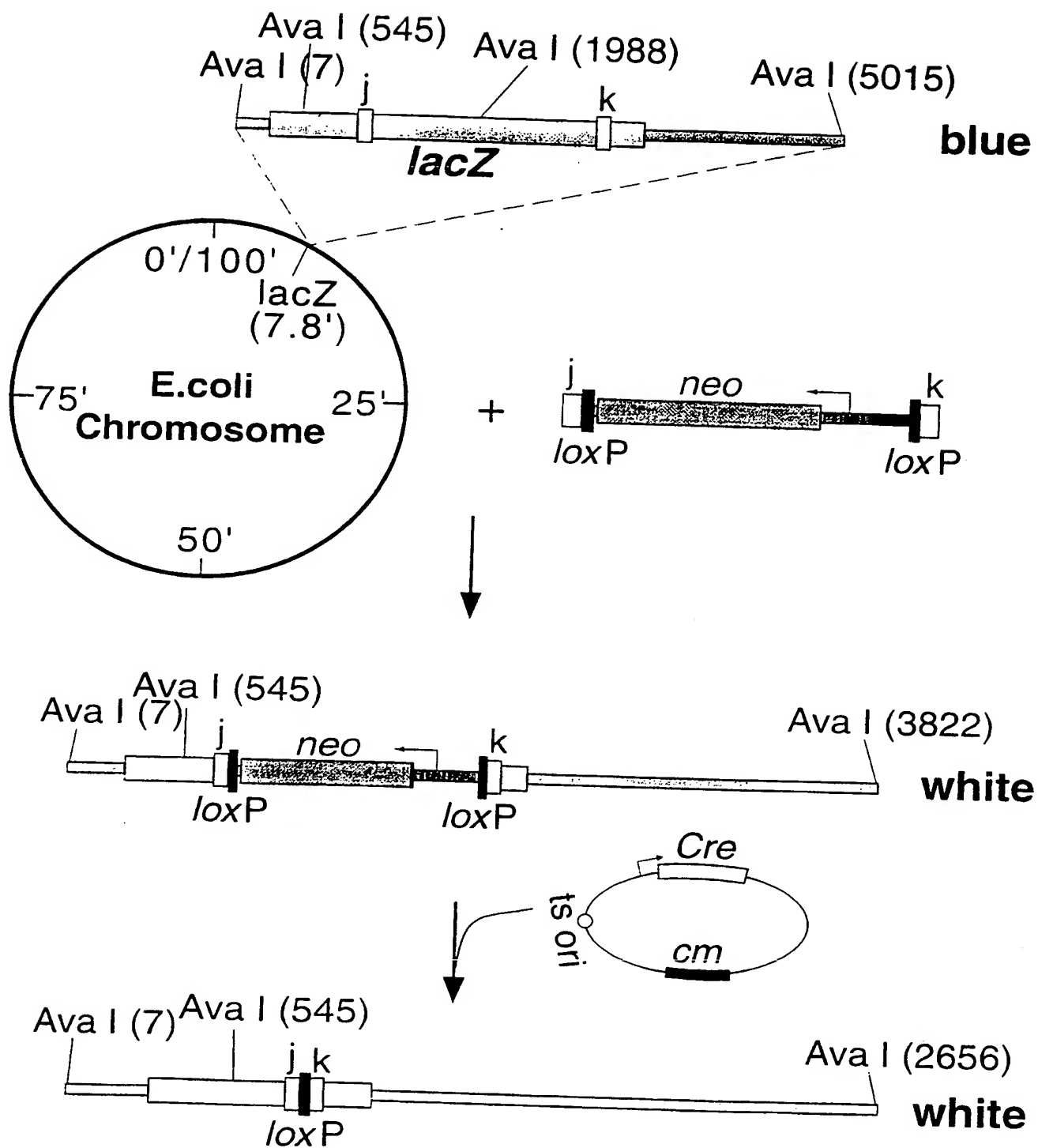


Figure 9

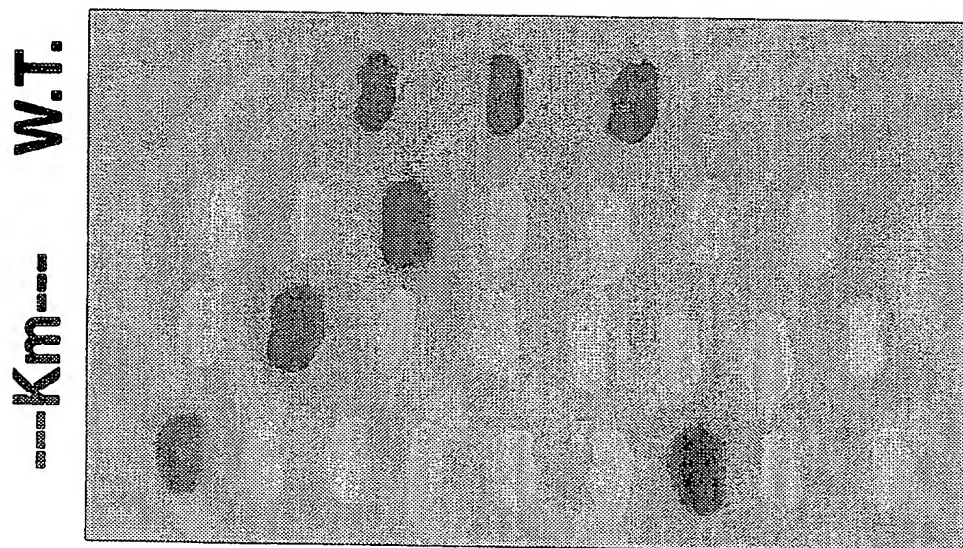
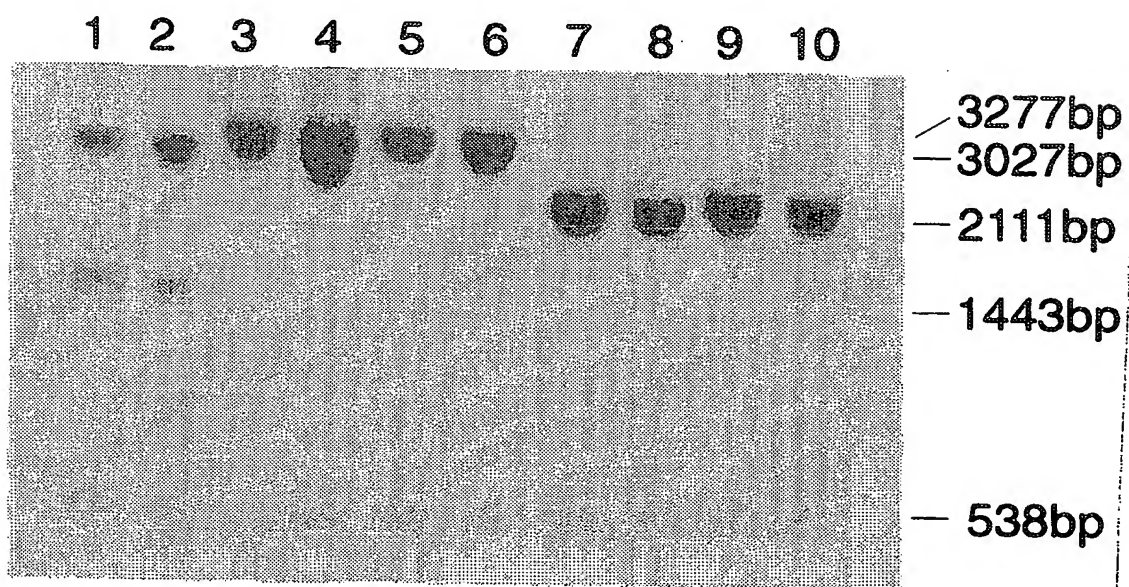
b*c*

Figure 10a

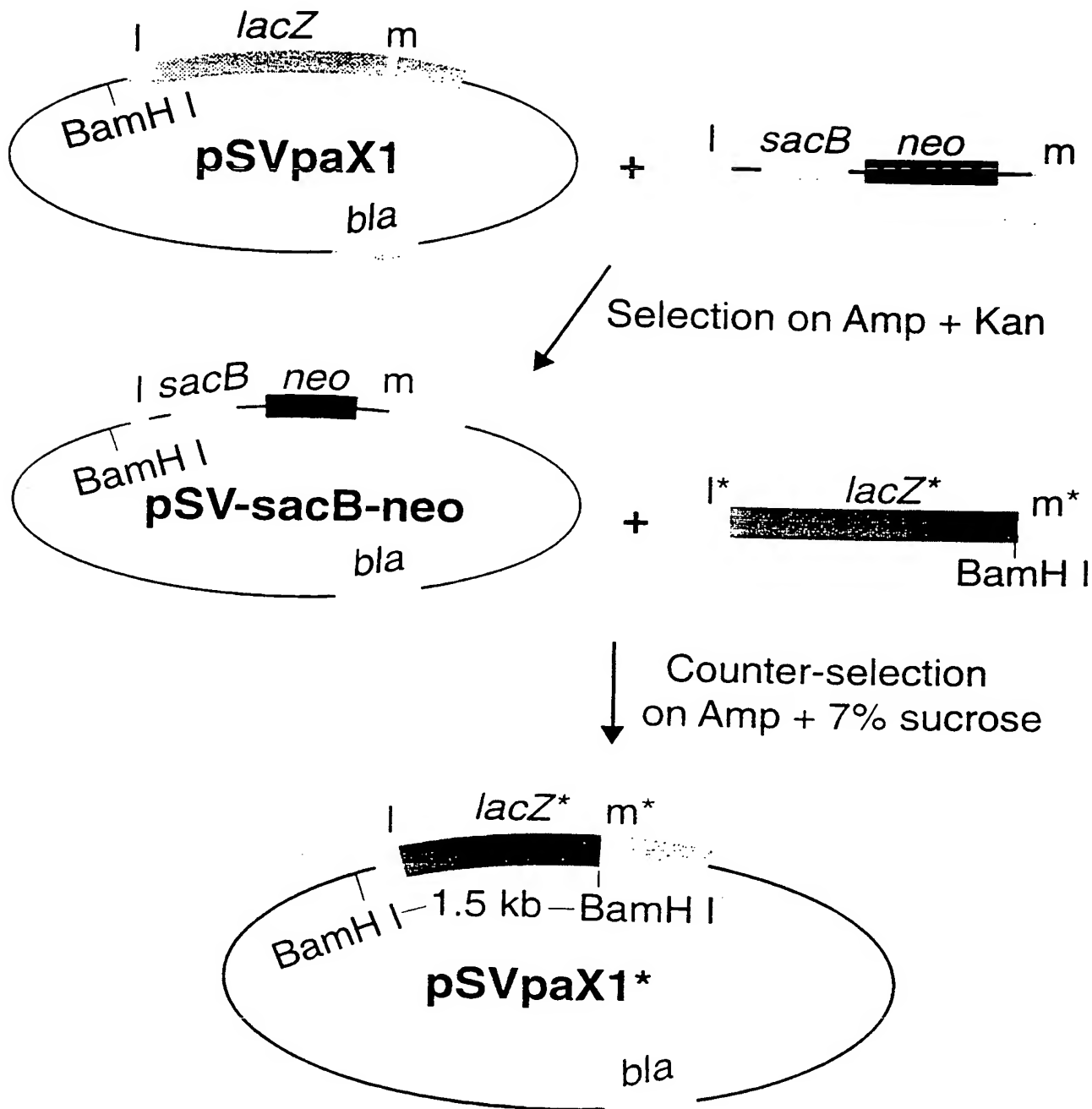


Figure 10

b

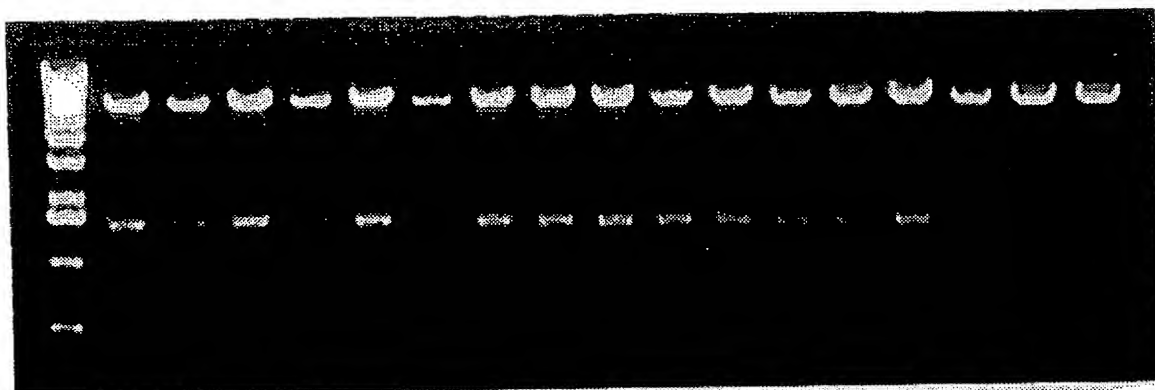
pSVpaX1

pSV-sacB-neo

pSVpaX1*

*c*

blue clones



white clones

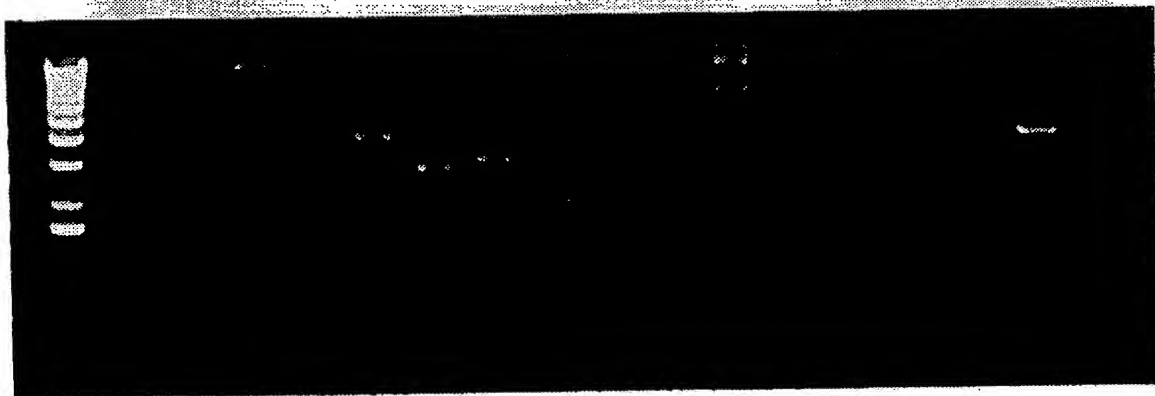


Figure 11a

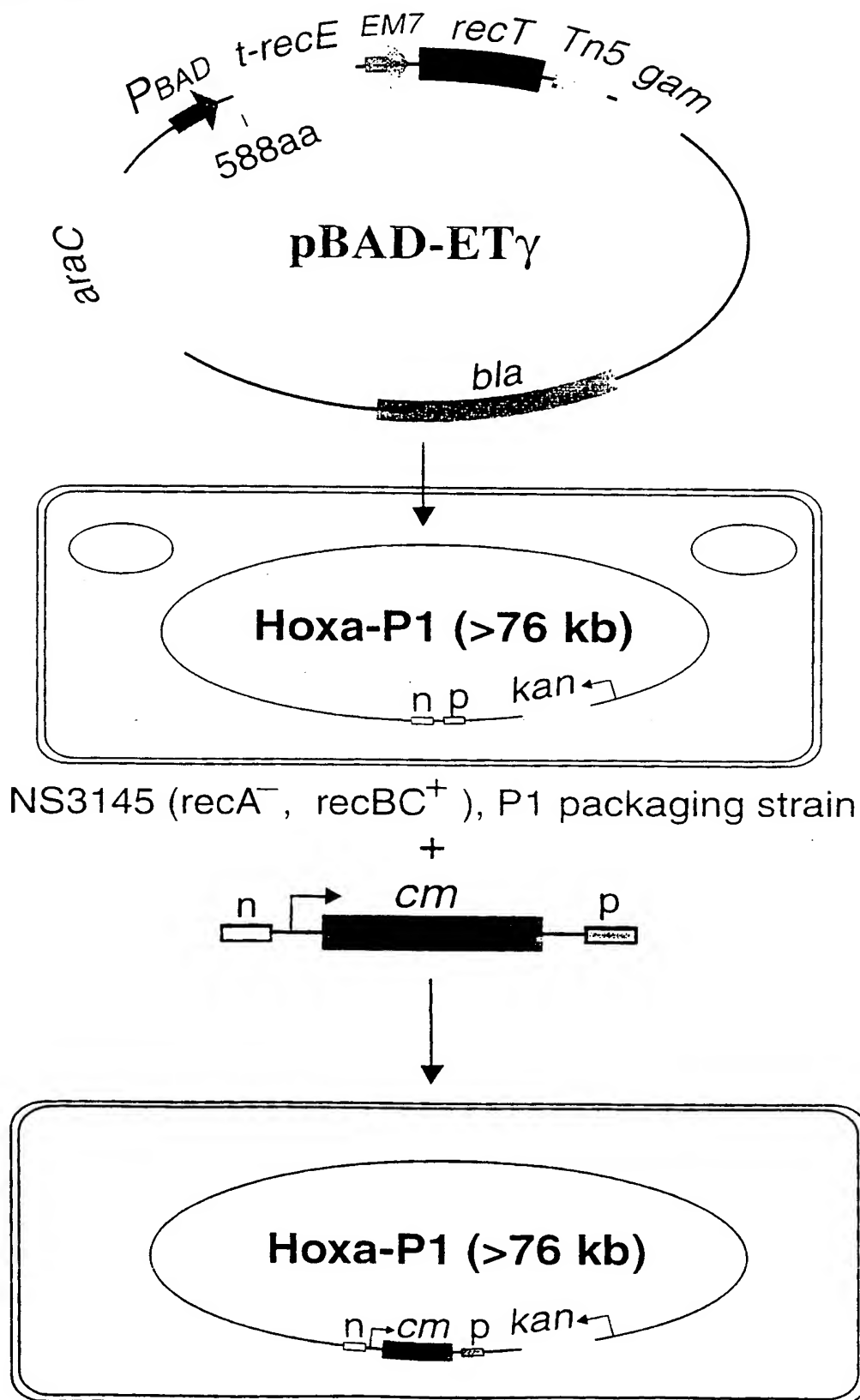


Figure 11 b

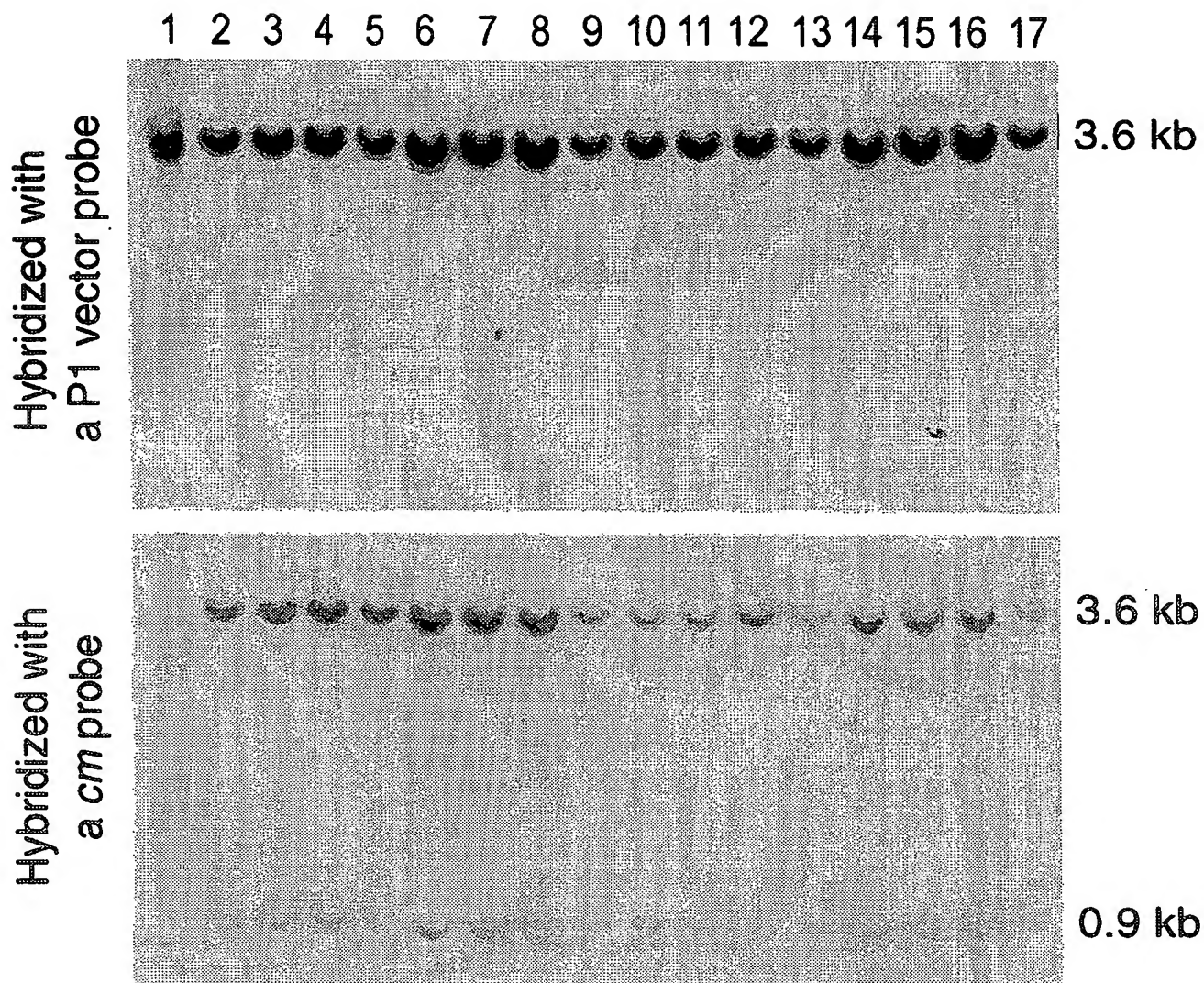


Figure 12

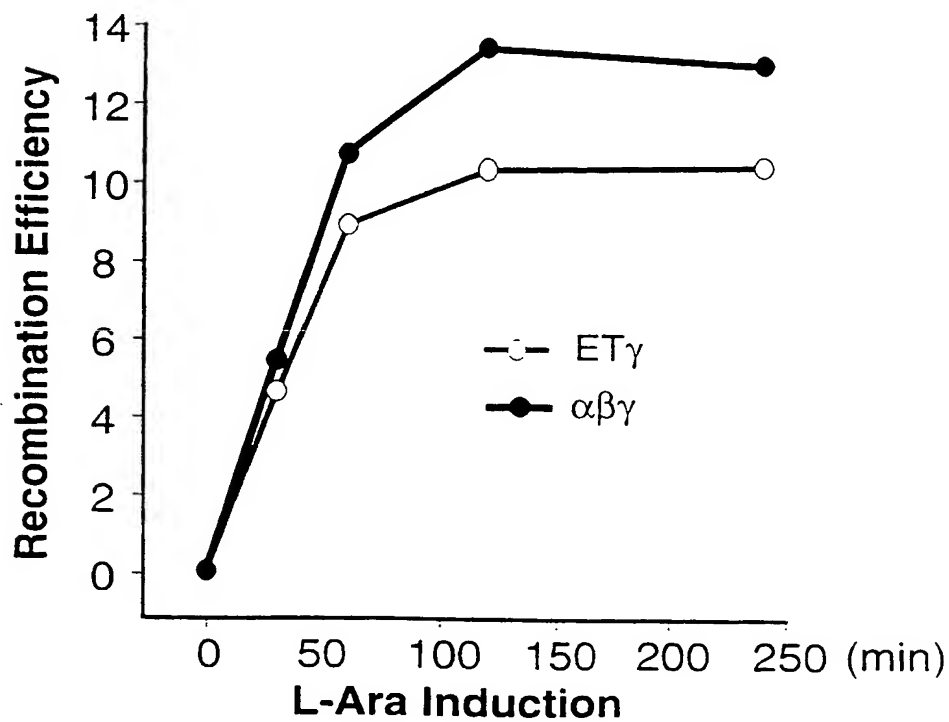
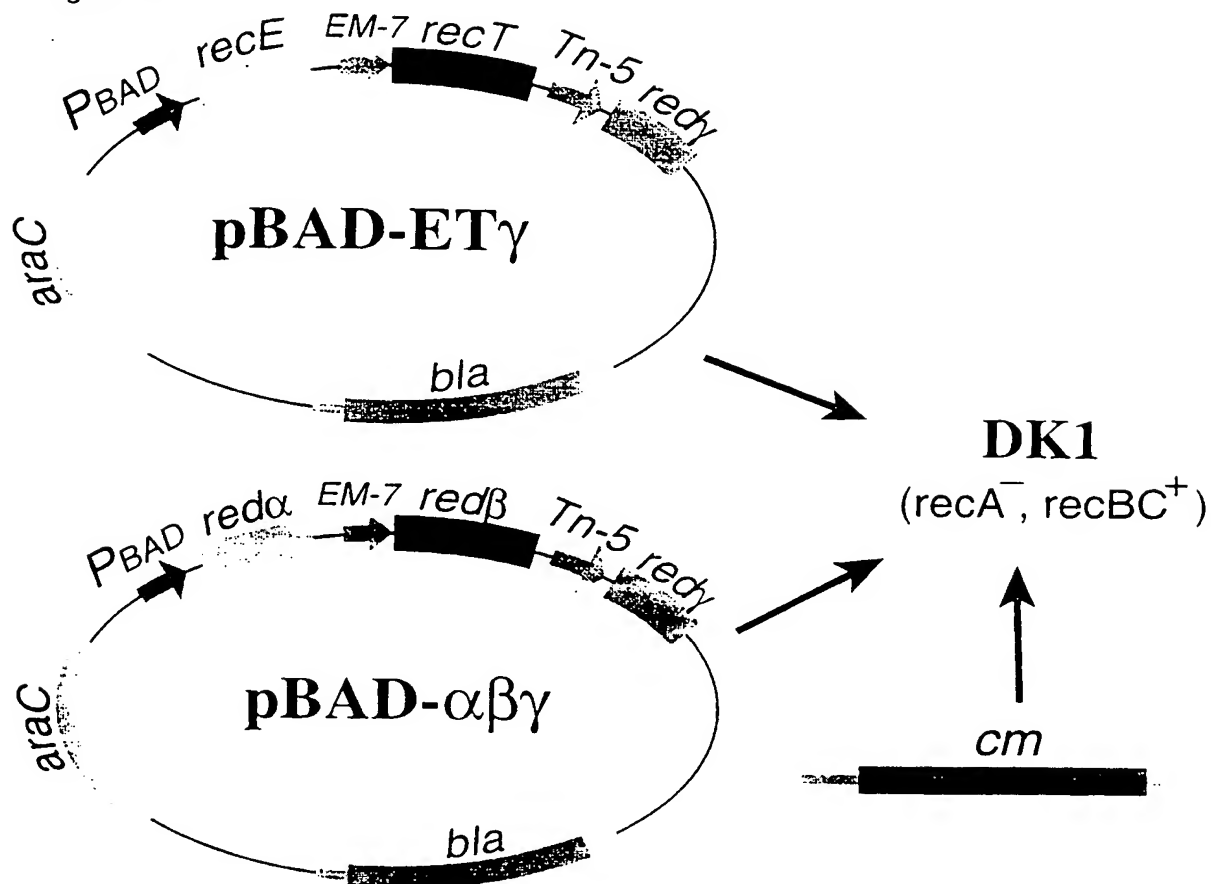
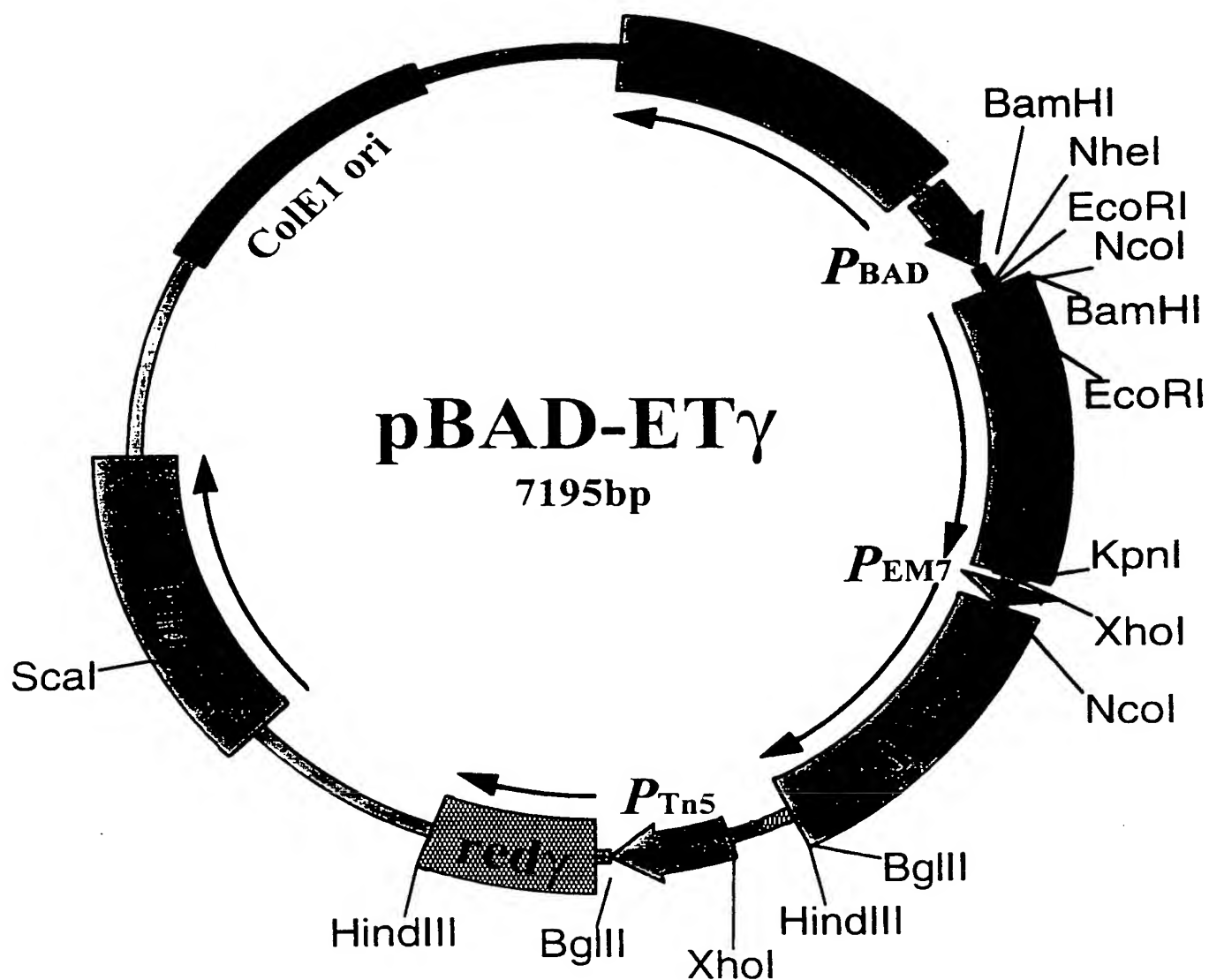


Figure 13 a



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Figure 13b

1 ATCGATGCATAATGTGCCTGTCAAATGGACGAAGCAGGG
40 ATTCTGCAAACCCTATGCTACTCCGTCAAGCCGTCAATT
79 GTCTGATTTCGTTACCAA TTA TGA CAA CTT GAC
293◀••• Ser Leu Lys Val

111 GGC TAC ATC ATT CAC TTT TTC TTC ACA ACC
288◀Ala Val Asp Asn Val Lys Glu Glu Cys Gly
141 GGC ACG GAA CTC GCT CGG GCT GGC CCC GGT
278◀Ala Arg Phe Glu Ser Pro Ser Ala Gly Thr
171 GCA TTT TTT AAA TAC CCG CGA GAA ATA GAG
268◀Cys Lys Lys Phe Val Arg Ser Phe Tyr Leu
201 TTG ATC GTC AAA ACC AAC ATT GCG ACC GAC
258◀Gln Asp Asp Phe Gly Val Asn Arg Gly Val
231 GGT GGC GAT AGG CAT CCG GGT GGT GCT CAA
248◀Thr Ala Ile Pro Met Arg Thr Thr Ser Leu
261 AAG CAG CTT CGC CTG GCT GAT ACG TTG GTC
238◀Leu Leu Lys Ala Gln Ser Ile Arg Gln Asp
291 CTC GCG CCA GCT TAA GAC GCT AAT CCC TAA
228◀Glu Arg Trp Ser Leu Val Ser Ile Gly Leu
321 CTG CTG GCG GAA AAG ATG TGA CAG ACG CGA
218◀Gln Gln Arg Phe Leu His Ser Leu Arg Ser
351 CGG CGA CAA GCA AAC ATG CTG TGC GAC GCT
208◀Pro Ser Leu Cys Val His Gln Ala Val Ser
381 GGC GAT ATC AAA ATT GCT GTC TGC CAG GTG
198◀Ala Ile Asp Phe Asn Ser Asp Ala Leu His
411 ATC GCT GAT GTA CTG ACA AGC CTC GCG TAC

Figure 13b (cont'd)

188	◀	Asp	Ser	Ile	Tyr	Gln	Cys	Ala	Glu	Arg	Val
441		CCG	ATT	ATC	CAT	CGG	TGG	ATG	GAG	CGA	CTC
178	◀	Arg	Asn	Asp	Met	Pro	Pro	His	Leu	Ser	Glu
471		GTT	AAT	CGC	TTC	CAT	GCG	CCG	CAG	TAA	CAA
168	◀	Asn	Ile	Ala	Glu	Met	Arg	Arg	Leu	Leu	Leu
501		TTG	CTC	AAG	CAG	ATT	TAT	CGC	CAG	CAG	CTC
158	◀	Gln	Glu	Leu	Leu	Asn	Ile	Ala	Leu	Leu	Glu
531		CGA	ATA	GCG	CCC	TTC	CCC	TTG	CCC	GGC	GTT
148	◀	Ser	Tyr	Arg	Gly	Glu	Gly	Gln	Gly	Ala	Asn
561		AAT	GAT	TTG	CCC	AAA	CAG	GTC	GCT	GAA	ATG
138	◀	Ile	Ile	Gln	Gly	Phe	Leu	Asp	Ser	Phe	His
591		CGG	CTG	GTG	CGC	TTC	ATC	CGG	GCG	AAA	GAA
128	◀	Pro	Gln	His	Ala	Glu	Asp	Pro	Arg	Phe	Phe
621		CCC	CGT	ATT	GGC	AAA	TAT	TGA	CGG	CCA	GTT
118	◀	Gly	Thr	Asn	Ala	Phe	Ile	Ser	Pro	Trp	Asn
651		AAG	CCA	TTC	ATG	CCA	GTA	GGC	GCG	CGG	ACG
108	◀	Leu	Trp	Glu	His	Trp	Tyr	Ala	Arg	Pro	Arg
681		AAA	GTA	AAC	CCA	CTG	GTG	ATA	CCA	TTC	GCG
98	◀	Phe	Tyr	Val	Trp	Gln	His	Tyr	Trp	Glu	Arg
711		AGC	CTC	CGG	ATG	ACG	ACC	GTA	GTG	ATG	AAT
88	◀	Ala	Glu	Pro	His	Arg	Gly	Tyr	His	His	Ile
741		CTC	TCC	TGG	CGG	GAA	CAG	CAA	AAT	ATC	ACC
78	◀	Glu	Gly	Pro	Pro	Phe	Leu	Leu	Ile	Asp	Gly
771		CGG	TCG	GCA	AAC	AAA	TTC	TCG	TCC	CTG	ATT
68	◀	Pro	Arg	Cys	Val	Phe	Glu	Arg	Gly	Gln	Asn

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Figure 13b (cont'd)

801 TTT CAC CAC CCC CTG ACC GCG AAT GGT GAG
58◀ Lys Val Val Gly Gln Gly Arg Ile Thr Leu

831 ATT GAG AAT ATA ACC TTT CAT TCC CAG CGG
48◀ Asn Leu Ile Tyr Gly Lys Met Gly Leu Pro

861 TCG GTC GAT AAA AAA ATC GAG ATA ACC GTT
38◀ Arg Asp Ile Phe Phe Asp Leu Tyr Gly Asn

891 GGC CTC AAT CGG CGT TAA ACC CGC CAC CAG
28◀ Ala Glu Ile Pro Thr Leu Gly Ala Val Leu

921 ATG GGC ATT AAA CGA GTA TCC CGG CAG CAG
18◀ His Ala Asn Phe Ser Tyr Gly Pro Leu Leu

951 GGG ATC ATT TTG CGC TTC AGC CAT ACTTTTC
8◀ Pro Asp Asn Gln Ala Glu Ala Met

982 ATACTCCCGCCATTTCAGAGAAGAAACCAATTGTCCATAT

1021 TGCATCAGACATTGCCGTCCTGCGTCTTTTACTGGCTC

1060 TTCTCGCTAACCAAACCGGTAACCCCGCTTATTAAAAGC

1099 ATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACG

1138 CGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCAC

1177 ATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATA

1216 GCATTTTTTATCCATAAGATTAGCGGATCCTACCTGACGC

BamHI



Figure 13b (cont'd)

1255 TTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTT
 NheI EcoRI NcoI BamHI

1294 TTTTGGGCTAGCAGGAGGAAT TCACC ATG GAT CCC
 1►Met Asp Pro

1329 GTA ATC GTA GAA GAC ATA GAG CCA GGT ATT
 4►Val Ile Val Glu Asp Ile Glu Pro Gly Ile

1359 TAT TAC GGA ATT TCG AAT GAG AAT TAC CAC
 14►Tyr Tyr Gly Ile Ser Asn Glu Asn Tyr His

1389 GCG GGT CCC GGT ATC AGT AAG TCT CAG CTC
 24►Ala Gly Pro Gly Ile Ser Lys Ser Gln Leu

1419 GAT GAC ATT GCT GAT ACT CCG GCA CTA TAT
 34►Asp Asp Ile Ala Asp Thr Pro Ala Leu Tyr

1449 TTG TGG CGT AAA AAT GCC CCC GTG GAC ACC
 44►Leu Trp Arg Lys Asn Ala Pro Val Asp Thr

1479 ACA AAG ACA AAA ACG CTC GAT TTA GGA ACT
 54►Thr Lys Thr Lys Thr Leu Asp Leu Gly Thr

1509 GCT TTC CAC TGC CGG GTA CTT GAA CCG GAA
 64►Ala Phe His Cys Arg Val Leu Glu Pro Glu

 EcoRI

1539 GAA TTC AGT AAC CGC TTT ATC GTA GCA CCT
 74►Glu Phe Ser Asn Arg Phe Ile Val Ala Pro

1569 GAA TTT AAC CGC CGT ACA AAC GCC GGA AAA
 84►Glu Phe Asn Arg Arg Thr Asn Ala Gly Lys

1599 GAA GAA GAG AAA GCG TTT CTG ATG GAA TGC
 94►Glu Glu Glu Lys Ala Phe Leu Met Glu Cys

1629 GCA AGC ACA GGA AAA ACG GTT ATC ACT GCG
 104►Ala Ser Thr Gly Lys Thr Val Ile Thr Ala

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Figure 13b (cont'd)

1659	GAA	GAA	GGC	CGG	AAA	ATT	GAA	CTC	ATG	TAT
114▶	Glu	Glu	Gly	Arg	Lys	Ile	Glu	Leu	Met	Tyr
1689	CAA	AGC	GTT	ATG	GCT	TTG	CCG	CTG	GGG	CAA
124▶	Gln	Ser	Val	Met	Ala	Leu	Pro	Leu	Gly	Gln
1719	TGG	CTT	GTT	GAA	AGC	GCC	GGA	CAC	GCT	GAA
134▶	Trp	Leu	Val	Glu	Ser	Ala	Gly	His	Ala	Glu
1749	TCA	TCA	ATT	TAC	TGG	GAA	GAT	CCT	GAA	ACA
144▶	Ser	Ser	Ile	Tyr	Trp	Glu	Asp	Pro	Glu	Thr
1779	GGA	ATT	TTG	TGT	CGG	TGC	CGT	CCG	GAC	AAA
154▶	Gly	Ile	Leu	Cys	Arg	Cys	Arg	Pro	Asp	Lys
1809	ATT	ATC	CCT	GAA	TTT	CAC	TGG	ATC	ATG	GAC
164▶	Ile	Ile	Pro	Glu	Phe	His	Trp	Ile	Met	Asp
1839	GTG	AAA	ACT	ACG	GCG	GAT	ATT	CAA	CGA	TTC
174▶	Val	Lys	Thr	Thr	Ala	Asp	Ile	Gln	Arg	Phe
1869	AAA	ACC	GCT	TAT	TAC	GAC	TAC	CGC	TAT	CAC
184▶	Lys	Thr	Ala	Tyr	Tyr	Asp	Tyr	Arg	Tyr	His
1899	GTT	CAG	GAT	GCA	TTC	TAC	AGT	GAC	GGT	TAT
194▶	Val	Gln	Asp	Ala	Phe	Tyr	Ser	Asp	Gly	Tyr
1929	GAA	GCA	CAG	TTT	GGA	GTG	CAG	CCA	ACT	TTC
204▶	Glu	Ala	Gln	Phe	Gly	Val	Gln	Pro	Thr	Phe
1959	GTT	TTT	CTG	GTT	GCC	AGC	ACA	ACT	ATT	GAA
214▶	Val	Phe	Leu	Val	Ala	Ser	Thr	Thr	Ile	Glu
1989	TGC	GGA	CGT	TAT	CCG	GTT	GAA	ATT	TTC	ATG
224▶	Cys	Gly	Arg	Tyr	Pro	Val	Glu	Ile	Phe	Met
2019	ATG	GGC	GAA	GAA	GCA	AAA	CTG	GCA	GGT	CAA
234▶	Met	Gly	Glu	Glu	Ala	Lys	Leu	Ala	Gly	Gln

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Figure 13b (cont'd)

2049 CAG GAA TAT CAC CGC AAT CTG CGA ACC CTG
244► Gln Glu Tyr His Arg Asn Leu Arg Thr Leu

2079 TCT GAC TGC CTG AAT ACC GAT GAA TGG CCA
254► Ser Asp Cys Leu Asn Thr Asp Glu Trp Pro

2109 GCT ATT AAG ACA TTA TCA CTG CCC CGC TGG
264► Ala Ile Lys Thr Leu Ser Leu Pro Arg Trp

XhoI KpnI

2139 GCT AAG GAA TAT GCA AAT GAC TAGATCTCGAG
274► Ala Lys Glu Tyr Ala Asn Asp

2171 GTACCCGAGCACGTGTTGACAATTAATCATCGGCATAGT

2210 ATATCGGCATAGTATAATACGACAAGGTGAGGAACTAAA
NcoI

2249 CC ATG GCT AAG CAA CCA CCA ATC GCA AAA
1► Met Ala Lys Gln Pro Pro Ile Ala Lys

2278 GCC GAT CTG CAA AAA ACT CAG GGA AAC CGT
10► Ala Asp Leu Gln Lys Thr Gln Gly Asn Arg

2308 GCA CCA GCA GCA GTT AAA AAT AGC GAC GTG
20► Ala Pro Ala Ala Val Lys Asn Ser Asp Val

2338 ATT AGT TTT ATT AAC CAG CCA TCA ATG AAA
30► Ile Ser Phe Ile Asn Gln Pro Ser Met Lys

2368 GAG CAA CTG GCA GCA GCT CTT CCA CGC CAT
40► Glu Gln Leu Ala Ala Ala Leu Pro Arg His

2398 ATG ACG GCT GAA CGT ATG ATC CGT ATC GCC
50► Met Thr Ala Glu Arg Met Ile Arg Ile Ala

2428 ACC ACA GAA ATT CGT AAA GTT CCG GCG TTA
60► Thr Thr Glu Ile Arg Lys Val Pro Ala Leu

Figure 13b (cont'd)

2458	GGA	AAC	TGT	GAC	ACT	ATG	AGT	TTT	GTC	AGT
70▶	Gly	Asn	Cys	Asp	Thr	Met	Ser	Phe	Val	Ser
2488	GCG	ATC	GTA	CAG	TGT	TCA	CAG	CTC	GGA	CTT
80▶	Ala	Ile	Val	Gln	Cys	Ser	Gln	Leu	Gly	Leu
2518	GAG	CCA	GGT	AGC	GCC	CTC	GGT	CAT	GCA	TAT
90▶	Glu	Pro	Gly	Ser	Ala	Leu	Gly	His	Ala	Tyr
2548	TTA	CTG	CCT	TTT	GGT	AAT	AAA	AAC	GAA	AAG
100▶	Leu	Leu	Pro	Phe	Gly	Asn	Lys	Asn	Glu	Lys
2578	AGC	GGT	AAA	AAG	AAC	GTT	CAG	CTA	ATC	ATT
110▶	Ser	Gly	Lys	Lys	Asn	Val	Gln	Leu	Ile	Ile
2608	GGC	TAT	CGC	GGC	ATG	ATT	GAT	CTG	GCT	CGC
120▶	Gly	Tyr	Arg	Gly	Met	Ile	Asp	Leu	Ala	Arg
2638	CGT	TCT	GGT	CAA	ATC	GCC	AGC	CTG	TCA	GCC
130▶	Arg	Ser	Gly	Gln	Ile	Ala	Ser	Leu	Ser	Ala
2668	CGT	GTT	GTC	CGT	GAA	GGT	GAC	GAG	TTT	AGC
140▶	Arg	Val	Val	Arg	Glu	Gly	Asp	Glu	Phe	Ser
2698	TTC	GAA	TTT	GGC	CTT	GAT	GAA	AAG	TTA	ATA
150▶	Phe	Glu	Phe	Gly	Leu	Asp	Glu	Lys	Leu	Ile
2728	CAC	CGC	CCG	GGA	GAA	AAC	GAA	GAT	GCC	CCG
160▶	His	Arg	Pro	Gly	Glu	Asn	Glu	Asp	Ala	Pro
2758	GTT	ACC	CAC	GTC	TAT	GCT	GTC	GCA	AGA	CTG
170▶	Val	Thr	His	Val	Tyr	Ala	Val	Ala	Arg	Leu
2788	AAA	GAC	GGA	GGT	ACT	CAG	TTT	GAA	GTT	ATG
180▶	Lys	Asp	Gly	Gly	Thr	Gln	Phe	Glu	Val	Met
2818	ACG	CGC	AAA	CAG	ATT	GAG	CTG	GTG	CGC	AGC
190▶	Thr	Arg	Lys	Gln	Ile	Glu	Leu	Val	Arg	Ser

Figure 13b (cont'd)

2848 CTG AGT AAA GCT GGT AAT AAC GGG CCG TGG
200▶ Leu Ser Lys Ala Gly Asn Asn Gly Pro Trp

2878 GTA ACT CAC TGG GAA GAA ATG GCA AAG AAA
210▶ Val Thr His Trp Glu Glu Met Ala Lys Lys

2908 ACG GCT ATT CGT CGC CTG TTC AAA TAT TTG
220▶ Thr Ala Ile Arg Arg Leu Phe Lys Tyr Leu

2938 CCC GTA TCA ATT GAG ATC CAG CGT GCA GTA
230▶ Pro Val Ser Ile Glu Ile Gln Arg Ala Val

2968 TCA ATG GAT GAA AAG GAA CCA CTG ACA ATC
240▶ Ser Met Asp Glu Lys Glu Pro Leu Thr Ile

2998 GAT CCT GCA GAT TCC TCT GTA TTA ACC GGG
250▶ Asp Pro Ala Asp Ser Ser Val Leu Thr Gly

3028 GAA TAC AGT GTA ATC GAT AAT TCA GAG GAA
260▶ Glu Tyr Ser Val Ile Asp Asn Ser Glu Glu

BglII HindIII

3058 TAG ATCTAAGCTTCCTGCTGAACATCAAAGGCAAGAAA
270▶ . . .

3096 ACATCTGTTGTCAAAGACAGCATCCTTGAACAAGGACAA

3135 TTAACAGTTAACAAATAAAAACGCAAAAGAAAATGCCGA

3174 TATCCTATTGGCATTTTCTTTTATTTCTTATCAACATAA

XhoI

3213 AGGTGAATCCCATACCTCGAGCTTCACGCTGCCGCAAGC

3252 ACTCAGGGCGCAAGGGCTGCTAAAAGGAAGCGGAACACG

3291 TAGAAAGCCAGTCCGCAGAAACGGTGCTGACCCCGGATG

3330 AATGTCAGCTACTGGGCTATCTGGACAAGGGAAAACGCA

3369 AGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGGGCTTACA

Figure 13b (cont'd)

3408 TGGCGATAGCTAGACTGGGCGGTTTATGGACAGCAAGC
3447 GAACCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTT
3486 GGG AAGCCCTGCAAAGTAAACTGGATGGCTTTCCTTGCCG
BglII
3525 CCAAGGATCTGATGGCGCAGGGGATCAAGATCTGATCAA
3564 GAGACAGGATGAGGATCGTTTCGC ATG GAT ATT
1►Met Asp Ile
3597 AAT ACT GAA ACT GAG ATC AAG CAA AAG CAT
4►Asn Thr Glu Thr Glu Ile Lys Gln Lys His
3627 TCA CTA ACC CCC TTT CCT GTT TTC CTA ATC
14►Ser Leu Thr Pro Phe Pro Val Phe Leu Ile
3657 AGC CCG GCA TTT CGC GGG CGA TAT TTT CAC
24►Ser Pro Ala Phe Arg Gly Arg Tyr Phe His
3687 AGC TAT TTC AGG AGT TCA GCC ATG AAC GCT
34►Ser Tyr Phe Arg Ser Ser Ala Met Asn Ala
3717 TAT TAC ATT CAG GAT CGT CTT GAG GCT CAG
44►Tyr Tyr Ile Gln Asp Arg Leu Glu Ala Gln
3747 AGC TGG GCG CGT CAC TAC CAG CAG CTC GCC
54►Ser Trp Ala Arg His Tyr Gln Gln Leu Ala
3777 CGT GAA GAG AAA GAG GCA GAA CTG GCA GAC
64►Arg Glu Glu Lys Glu Ala Glu Leu Ala Asp
3807 GAC ATG GAA AAA GGC CTG CCC CAG CAC CTG
74►Asp Met Glu Lys Gly Leu Pro Gln His Leu
3837 TTT GAA TCG CTA TGC ATC GAT CAT TTG CAA
84►Phe Glu Ser Leu Cys Ile Asp His Leu Gln
3867 CGC CAC GGG GCC AGC AAA AAA TCC ATT ACC
94►Arg His Gly Ala Ser Lys Lys Ser Ile Thr

Figure 13b (cont'd)

3897 CGT GCG TTT GAT GAC GAT GTT GAG TTT CAG
104▶ Arg Ala Phe Asp Asp Asp Val Glu Phe Gln

3927 GAG CGC ATG GCA GAA CAC ATC CGG TAC ATG
114▶ Glu Arg Met Ala Glu His Ile Arg Tyr Met

3957 GTT GAA ACC ATT GCT CAC CAC CAG GTT GAT
124▶ Val Glu Thr Ile Ala His His Gln Val Asp
HindIII

3987 ATT GAT TCA GAG GTA TAA AACGAGTAGA AGCT
134▶ Ile Asp Ser Glu Val ...

4019 TGGCTGTTTTGGCGGATGAGAGAAGATTTTCAGCCTGAT
4058 ACAGATTAAATCAGAACGCAGAAGCGGTCTGATAAAACA
4097 GAATTTGCCTGGCGGCAGTAGCGCGGTGGTCCCACCTGA
4136 CCCCATGCCGAACCTCAGAAGTGAAACGCCGTAGCGCCGA
4175 TGGTAGTGTGGGGTCTCCCCATGCGAGAGTAGGGAACTG
4214 CCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT
4253 GGGCCTTTTCGTTTTATCTGTTGTTTGTTCGGTGAACGCTC
4292 TCCTGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACG
4331 TTGCGAAGCAACGGCCCCGAGGGTGGCGGGCAGGACGCC
4370 CGCCATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCA
4409 TCCTGACGGATGGCCTTTTTTGCGTTTCTACAAACTCTTT
4448 TGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTC
4487 ATGAGACAATAACCCTGATAAATGCTTCAATAATATTGA
4526 AAAAGGAAGAGT ATG AGT ATT CAA CAT TTC
1▶ Met Ser Ile Gln His Phe

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Figure 13b (cont'd)

4556	CGT	GTC	GCC	CTT	ATT	CCC	TTT	TTT	GCG	GCA
7▶	Arg	Val	Ala	Leu	Ile	Pro	Phe	Phe	Ala	Ala
4586	TTT	TGC	CTT	CCT	GTT	TTT	GCT	CAC	CCA	GAA
17▶	Phe	Cys	Leu	Pro	Val	Phe	Ala	His	Pro	Glu
4616	ACG	CTG	GTG	AAA	GTA	AAA	GAT	GCT	GAA	GAT
27▶	Thr	Leu	Val	Lys	Val	Lys	Asp	Ala	Glu	Asp
4646	CAG	TTG	GGT	GCA	CGA	GTG	GGT	TAC	ATC	GAA
37▶	Gln	Leu	Gly	Ala	Arg	Val	Gly	Tyr	Ile	Glu
4676	CTG	GAT	CTC	AAC	AGC	GGT	AAG	ATC	CTT	GAG
47▶	Leu	Asp	Leu	Asn	Ser	Gly	Lys	Ile	Leu	Glu
4706	AGT	TTT	CGC	CCC	GAA	GAA	CGT	TTT	CCA	ATG
57▶	Ser	Phe	Arg	Pro	Glu	Glu	Arg	Phe	Pro	Met
4736	ATG	AGC	ACT	TTT	AAA	GTT	CTG	CTA	TGT	GGC
67▶	Met	Ser	Thr	Phe	Lys	Val	Leu	Leu	Cys	Gly
4766	GCG	GTA	TTA	TCC	CGT	GTT	GAC	GCC	GGG	CAA
77▶	Ala	Val	Leu	Ser	Arg	Val	Asp	Ala	Gly	Gln
4796	GAG	CAA	CTC	GGT	CGC	CGC	ATA	CAC	TAT	TCT
87▶	Glu	Gln	Leu	Gly	Arg	Arg	Ile	His	Tyr	Ser
							Scal			
4826	CAG	AAT	GAC	TTG	GTT	GAG	TAC	TCA	CCA	GTC
97▶	Gln	Asn	Asp	Leu	Val	Glu	Tyr	Ser	Pro	Val
4856	ACA	GAA	AAG	CAT	CTT	ACG	GAT	GGC	ATG	ACA
107▶	Thr	Glu	Lys	His	Leu	Thr	Asp	Gly	Met	Thr
4886	GTA	AGA	GAA	TTA	TGC	AGT	GCT	GCC	ATA	ACC
117▶	Val	Arg	Glu	Leu	Cys	Ser	Ala	Ala	Ile	Thr
4916	ATG	AGT	GAT	AAC	ACT	GCG	GCC	AAC	TTA	CTT
127▶	Met	Ser	Asp	Asn	Thr	Ala	Ala	Asn	Leu	Leu

Figure 13b (cont'd)

4946	CTG	ACA	ACG	ATC	GGA	GGA	CCG	AAG	GAG	CTA
137▶	Leu	Thr	Thr	Ile	Gly	Gly	Pro	Lys	Glu	Leu
4976	ACC	GCT	TTT	TTG	CAC	AAC	ATG	GGG	GAT	CAT
147▶	Thr	Ala	Phe	Leu	His	Asn	Met	Gly	Asp	His
5006	GTA	ACT	CGC	CTT	GAT	CGT	TGG	GAA	CCG	GAG
157▶	Val	Thr	Arg	Leu	Asp	Arg	Trp	Glu	Pro	Glu
5036	CTG	AAT	GAA	GCC	ATA	CCA	AAC	GAC	GAG	CGT
167▶	Leu	Asn	Glu	Ala	Ile	Pro	Asn	Asp	Glu	Arg
5066	GAC	ACC	ACG	ATG	CCT	GTA	GCA	ATG	GCA	ACA
177▶	Asp	Thr	Thr	Met	Pro	Val	Ala	Met	Ala	Thr
5096	ACG	TTG	CGC	AAA	CTA	TTA	ACT	GGC	GAA	CTA
187▶	Thr	Leu	Arg	Lys	Leu	Leu	Thr	Gly	Glu	Leu
5126	CTT	ACT	CTA	GCT	TCC	CGG	CAA	CAA	TTA	ATA
197▶	Leu	Thr	Leu	Ala	Ser	Arg	Gln	Gln	Leu	Ile
5156	GAC	TGG	ATG	GAG	GCG	GAT	AAA	GTT	GCA	GGA
207▶	Asp	Trp	Met	Glu	Ala	Asp	Lys	Val	Ala	Gly
5186	CCA	CTT	CTG	CGC	TCG	GCC	CTT	CCG	GCT	GGC
217▶	Pro	Leu	Leu	Arg	Ser	Ala	Leu	Pro	Ala	Gly
5216	TGG	TTT	ATT	GCT	GAT	AAA	TCT	GGA	GCC	GGT
227▶	Trp	Phe	Ile	Ala	Asp	Lys	Ser	Gly	Ala	Gly
5246	GAG	CGT	GGG	TCT	CGC	GGT	ATC	ATT	GCA	GCA
237▶	Glu	Arg	Gly	Ser	Arg	Gly	Ile	Ile	Ala	Ala
5276	CTG	GGG	CCA	GAT	GGT	AAG	CCC	TCC	CGT	ATC
247▶	Leu	Gly	Pro	Asp	Gly	Lys	Pro	Ser	Arg	Ile
5306	GTA	GTT	ATC	TAC	ACG	ACG	GGG	AGT	CAG	GCA
257▶	Val	Val	Ile	Tyr	Thr	Thr	Gly	Ser	Gln	Ala

Figure 13b (cont'd)

5336 ACT ATG GAT GAA CGA AAT AGA CAG ATC GCT
267▶ Thr Met Asp Glu Arg Asn Arg Gln Ile Ala
5366 GAG ATA GGT GCC TCA CTG ATT AAG CAT TGG
277▶ Glu Ile Gly Ala Ser Leu Ile Lys His Trp
5396 TAA CTGTCAGACCAAGTTTACTCATATATACTTTAGAT
287▶ . . .

5434 TGATTTACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGG
5473 GTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCA
5512 GCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCCT
5551 TTCTCGCCACGTTTCGCCGGCTTTCCCCGTCAAGCTCTAA
5590 ATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTAC
5629 GGCACCTCGACCCCAAAAACTTGATTTGGGTGATGGTT
5668 CACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCC
5707 CTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCT
5746 TGTTCCAAACTTGAACAACACTCAACCCTATCTCGGGCT
5785 ATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCGGCCT
5824 ATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACG
5863 CGAATTTTAACAAAATATTAACGTTTACAATTTAAAAGG
5902 ATCTAGGTGAAGATCCTTTTGTGATAATCTCATGACCAAA
5941 ATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGAC
5980 CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTT
6019 TTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCA
6058 CCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTA
6097 CCAACTCTTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCG

Figure 13b (cont'd)

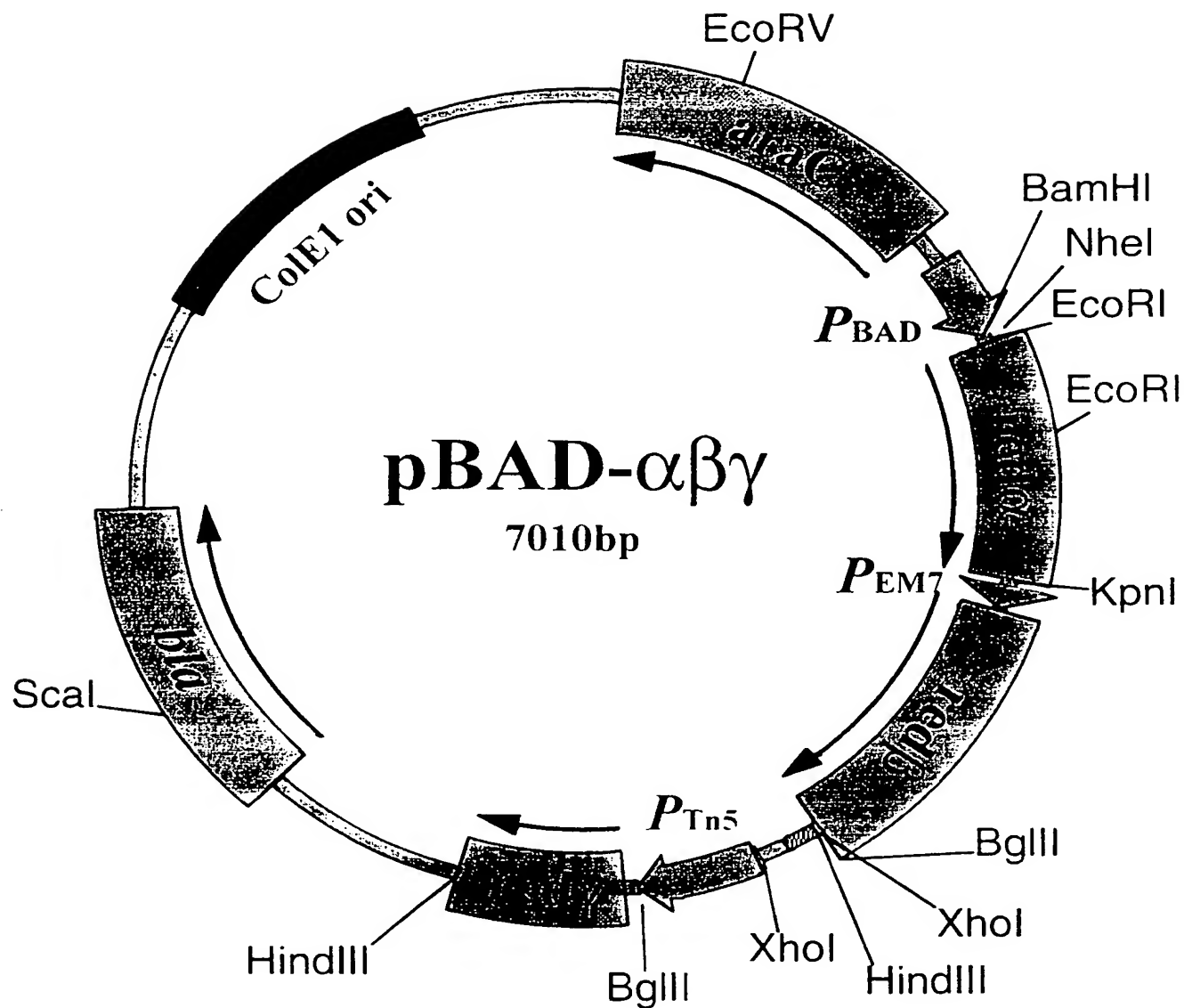
6136 CAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTA
6175 GGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATAC
6214 CTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGT
6253 GGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGA
6292 TAGTTACCGGATAAGGCGCAGCGGTCTGGGCTGAACGGGG
6331 GGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTAC
6370 ACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGC
6409 GCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCG
6448 GTAAGCGGCAGGGTCTGGAACAGGAGAGCGCACGAGGGAG
6487 CTTCCAGGGGGGAAACGCCTGGTATCTTTATAGTCCTGTC
6526 GGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTTGTGA
6565 TGCTCGTCAGGGGGGGCGGAGCCTATGGAAAAACGCCAGC
6604 AACGCGGCCTTTTTTACGGTTCCTGGCCTTTTGTCTGGCCT
6643 TTTGCTCACATGTTCTTTTCCTGCGTTATCCCCTGATTCT
6682 GTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACC
6721 GCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTG
6760 AGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTC
6799 CTTACGCATCTGTGCGGTATTTTCACACCGCATAGGGTCA
6838 TGGCTGCGCCCCGACACCCGCCAACACCCGCTGACGCGC
6877 CCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGAC
6916 AAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGT
6955 TTTCACCGTCATCACCGAAACGCGCGAGGCAGCAAGGAG

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Figure 13b (cont'd)

6994 ATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGCCACC
7033 ATACCCACGCCGAAACAAGCGCTCATGAGCCCGAAGTGG
7072 CGAGCCCGATCTTCCCCATCGGTGATGTCGGCGATATAG
7111 GCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCCGGCC
7150 ACGATGCGTCCGGCGTAGAGGATCTGCTCATGTTTGACA
7189 GCTTATC

Figure 14 a



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Figure 14b

NsiI

1 ATCGATGCATAATGTGCCTGTCAAATGGACGAAGCAGGG
 40 ATTCTGCAAACCCTATGCTACTCCGTCAAGCCGTCAATT
 79 GTCTGATTTCGTTACCAA TTA TGA CAA CTT GAC
 293◀••• Ser Leu Lys Val

 111 GGC TAC ATC ATT CAC TTT TTC TTC ACA ACC
 288◀Ala Val Asp Asn Val Lys Glu Glu Cys Gly
 141 GGC ACG GAA CTC GCT CGG GCT GGC CCC GGT
 278◀Ala Arg Phe Glu Ser Pro Ser Ala Gly Thr
 171 GCA TTT TTT AAA TAC CCG CGA GAA ATA GAG
 268◀Cys Lys Lys Phe Val Arg Ser Phe Tyr Leu
 201 TTG ATC GTC AAA ACC AAC ATT GCG ACC GAC
 258◀Gln Asp Asp Phe Gly Val Asn Arg Gly Val
 231 GGT GGC GAT AGG CAT CCG GGT GGT GCT CAA
 248◀Thr Ala Ile Pro Met Arg Thr Thr Ser Leu
 261 AAG CAG CTT CGC CTG GCT GAT ACG TTG GTC
 238◀Leu Leu Lys Ala Gln Ser Ile Arg Gln Asp
 291 CTC GCG CCA GCT TAA GAC GCT AAT CCC TAA
 228◀Glu Arg Trp Ser Leu Val Ser Ile Gly Leu
 321 CTG CTG GCG GAA AAG ATG TGA CAG ACG CGA
 218◀Gln Gln Arg Phe Leu His Ser Leu Arg Ser
 351 CGG CGA CAA GCA AAC ATG CTG TGC GAC GCT
 208◀Pro Ser Leu Cys Val His Gln Ala Val Ser
 EcoRV
 381 GGC GAT ATC AAA ATT GCT GTC TGC CAG GTG
 198◀Ala Ile Asp Phe Asn Ser Asp Ala Leu His

Figure 14b (cont'd)

411	ATC	GCT	GAT	GTA	CTG	ACA	AGC	CTC	GCG	TAC
188	◀Asp	Ser	Ile	Tyr	Gln	Cys	Ala	Glu	Arg	Val
441	CCG	ATT	ATC	CAT	CGG	TGG	ATG	GAG	CGA	CTC
178	◀Arg	Asn	Asp	Met	Pro	Pro	His	Leu	Ser	Glu
471	GTT	AAT	CGC	TTC	CAT	GCG	CCG	CAG	TAA	CAA
168	◀Asn	Ile	Ala	Glu	Met	Arg	Arg	Leu	Leu	Leu
501	TTG	CTC	AAG	CAG	ATT	TAT	CGC	CAG	CAG	CTC
158	◀Gln	Glu	Leu	Leu	Asn	Ile	Ala	Leu	Leu	Glu
531	CGA	ATA	GCG	CCC	TTC	CCC	TTG	CCC	GGC	GTT
148	◀Ser	Tyr	Arg	Gly	Glu	Gly	Gln	Gly	Ala	Asn
561	AAT	GAT	TTG	CCC	AAA	CAG	GTC	GCT	GAA	ATG
138	◀Ile	Ile	Gln	Gly	Phe	Leu	Asp	Ser	Phe	His
591	CGG	CTG	GTG	CGC	TTC	ATC	CGG	GCG	AAA	GAA
128	◀Pro	Gln	His	Ala	Glu	Asp	Pro	Arg	Phe	Phe
621	CCC	CGT	ATT	GGC	AAA	TAT	TGA	CGG	CCA	GTT
118	◀Gly	Thr	Asn	Ala	Phe	Ile	Ser	Pro	Trp	Asn
651	AAG	CCA	TTC	ATG	CCA	GTA	GGC	GCG	CGG	ACG
108	◀Leu	Trp	Glu	His	Trp	Tyr	Ala	Arg	Pro	Arg
681	AAA	GTA	AAC	CCA	CTG	GTG	ATA	CCA	TTC	GCG
98	◀Phe	Tyr	Val	Trp	Gln	His	Tyr	Trp	Glu	Arg
711	AGC	CTC	CGG	ATG	ACG	ACC	GTA	GTG	ATG	AAT
88	◀Ala	Glu	Pro	His	Arg	Gly	Tyr	His	His	Ile
741	CTC	TCC	TGG	CGG	GAA	CAG	CAA	AAT	ATC	ACC
78	◀Glu	Gly	Pro	Pro	Phe	Leu	Leu	Ile	Asp	Gly
771	CGG	TCG	GCA	AAC	AAA	TTC	TCG	TCC	CTG	ATT
68	◀Pro	Arg	Cys	Val	Phe	Glu	Arg	Gly	Gln	Asn

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Figure 14b (cont'd)

801 TTT CAC CAC CCC CTG ACC GCG AAT GGT GAG
58◀Lys Val Val Gly Gln Gly Arg Ile Thr Leu

831 ATT GAG AAT ATA ACC TTT CAT TCC CAG CGG
48◀Asn Leu Ile Tyr Gly Lys Met Gly Leu Pro

861 TCG GTC GAT AAA AAA ATC GAG ATA ACC GTT
38◀Arg Asp Ile Phe Phe Asp Leu Tyr Gly Asn

891 GGC CTC AAT CGG CGT TAA ACC CGC CAC CAG
28◀Ala Glu Ile Pro Thr Leu Gly Ala Val Leu

921 ATG GGC ATT AAA CGA GTA TCC CGG CAG CAG
18◀His Ala Asn Phe Ser Tyr Gly Pro Leu Leu

951 GGG ATC ATT TTG CGC TTC AGC CAT ACTTTTC
8◀Pro Asp Asn Gln Ala Glu Ala Met

982 ATACTCCCGCCATTCAGAGAAGAAACCAATTGTCCATAT

1021 TGCATCAGACATTGCCGTCCTGCGTCTTTTACTGGCTC

1060 TTCTCGCTAACCAAACCGGTAACCCCGCTTATTAAAAGC

1099 ATTCTGTAACAAAGCGGGACCAAAGCCATGACAAAAACG

1138 CGTAACAAAAGTGTCTATAATCACGGCAGAAAAGTCCAC

1177 ATTGATTATTTGCACGGCGTCACACTTTGCTATGCCATA
BamHI

1216 GCATTTTTTATCCATAAGATTAGCGGATCCTACCTGACGC

1255 TTTTTATCGCAACTCTCTACTGTTTCTCCATACCCGTTT
NheI EcoRI

1294 TTTTGGGCTAGCAGGAGGAATTCACC ATG ACA CCG
1▶Met Thr Pro

PstI

1329 GAC ATT ATC CTG CAG CGT ACC GGG ATC GAT

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Figure 14b (cont'd)

	4▶	Asp	Ile	Ile	Leu	Gln	Arg	Thr	Gly	Ile	Asp
1359		GTG	AGA	GCT	GTC	GAA	CAG	GGG	GAT	GAT	GCG
	14▶	Val	Arg	Ala	Val	Glu	Gln	Gly	Asp	Asp	Ala
1389		TGG	CAC	AAA	TTA	CGG	CTC	GGC	GTC	ATC	ACC
	24▶	Trp	His	Lys	Leu	Arg	Leu	Gly	Val	Ile	Thr
1419		GCT	TCA	GAA	GTT	CAC	AAC	GTG	ATA	GCA	AAA
	34▶	Ala	Ser	Glu	Val	His	Asn	Val	Ile	Ala	Lys
1449		CCC	CGC	TCC	GGA	AAG	AAG	TGG	CCT	GAC	ATG
	44▶	Pro	Arg	Ser	Gly	Lys	Lys	Trp	Pro	Asp	Met
1479		AAA	ATG	TCC	TAC	TTC	CAC	ACC	CTG	CTT	GCT
	54▶	Lys	Met	Ser	Tyr	Phe	His	Thr	Leu	Leu	Ala
1509		GAG	GTT	TGC	ACC	GGT	GTG	GCT	CCG	GAA	GTT
	64▶	Glu	Val	Cys	Thr	Gly	Val	Ala	Pro	Glu	Val
1539		AAC	GCT	AAA	GCA	CTG	GCC	TGG	GGA	AAA	CAG
	74▶	Asn	Ala	Lys	Ala	Leu	Ala	Trp	Gly	Lys	Gln
											EcoRI
1569		TAC	GAG	AAC	GAC	GCC	AGA	ACC	CTG	TTT	GAA
	84▶	Tyr	Glu	Asn	Asp	Ala	Arg	Thr	Leu	Phe	Glu
1599		TTC	ACT	TCC	GGC	GTG	AAT	GTT	ACT	GAA	TCC
	94▶	Phe	Thr	Ser	Gly	Val	Asn	Val	Thr	Glu	Ser
1629		CCG	ATC	ATC	TAT	CGC	GAC	GAA	AGT	ATG	CGT
	104▶	Pro	Ile	Ile	Tyr	Arg	Asp	Glu	Ser	Met	Arg
1659		ACC	GCC	TGC	TCT	CCC	GAT	GGT	TTA	TGC	AGT
	114▶	Thr	Ala	Cys	Ser	Pro	Asp	Gly	Leu	Cys	Ser
1689		GAC	GGC	AAC	GGC	CTT	GAA	CTG	AAA	TGC	CCG
	124▶	Asp	Gly	Asn	Gly	Leu	Glu	Leu	Lys	Cys	Pro

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Figure 14b (cont'd)

1719 TTT ACC TCC CGG GAT TTC ATG AAG TTC CGG
 134▶ Phe Thr Ser Arg Asp Phe Met Lys Phe Arg
 1749 CTC GGT GGT TTC GAG GCC ATA AAG TCA GCT
 144▶ Leu Gly Gly Phe Glu Ala Ile Lys Ser Ala
 1779 TAC ATG GCC CAG GTG CAG TAC AGC ATG TGG
 154▶ Tyr Met Ala Gln Val Gln Tyr Ser Met Trp
 1809 GTG ACG CGA AAA AAT GCC TGG TAC TTT GCC
 164▶ Val Thr Arg Lys Asn Ala Trp Tyr Phe Ala
 1839 AAC TAT GAC CCG CGT ATG AAG CGT GAA GGC
 174▶ Asn Tyr Asp Pro Arg Met Lys Arg Glu Gly
 1869 CTG CAT TAT GTC GTG ATT GAG CGG GAT GAA
 184▶ Leu His Tyr Val Val Ile Glu Arg Asp Glu
 1899 AAG TAC ATG GCG AGT TTT GAC GAG ATC GTG
 194▶ Lys Tyr Met Ala Ser Phe Asp Glu Ile Val
 1929 CCG GAG TTC ATC GAA AAA ATG GAC GAG GCA
 204▶ Pro Glu Phe Ile Glu Lys Met Asp Glu Ala
 1959 CTG GCT GAA ATT GGT TTT GTA TTT GGG GAG
 214▶ Leu Ala Glu Ile Gly Phe Val Phe Gly Glu
 KpnI
 1989 CAA TGG CGA TAGATCCGGTACCCGAGCACGTGTGA
 224▶ Gln Trp Arg . . .
 2025 CAATTAATCATCGGCATAGTATATCGGCATAGTATAATA
 2064 CGACAAGGTGAGGAACTAAACC ATG AGT ACT GCA
 1▶ Met Ser Thr Ala
 2098 CTC GCA ACG CTG GCT GGG AAG CTG GCT GAA
 5▶ Leu Ala Thr Leu Ala Gly Lys Leu Ala Glu

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Figure 14b (cont'd)

											Sall
2128	CGT	GTC	GGC	ATG	GAT	TCT	GTC	GAC	CCA	CAG	
15▶	Arg	Val	Gly	Met	Asp	Ser	Val	Asp	Pro	Gln	
2158	GAA	CTG	ATC	ACC	ACT	CTT	CGC	CAG	ACG	GCA	
25▶	Glu	Leu	Ile	Thr	Thr	Leu	Arg	Gln	Thr	Ala	
2188	TTT	AAA	GGT	GAT	GCC	AGC	GAT	GCG	CAG	TTC	
35▶	Phe	Lys	Gly	Asp	Ala	Ser	Asp	Ala	Gln	Phe	
2218	ATC	GCA	TTA	CTG	ATC	GTT	GCC	AAC	CAG	TAC	
45▶	Ile	Ala	Leu	Leu	Ile	Val	Ala	Asn	Gln	Tyr	
2248	GGC	CTT	AAT	CCG	TGG	ACG	AAA	GAA	ATT	TAC	
55▶	Gly	Leu	Asn	Pro	Trp	Thr	Lys	Glu	Ile	Tyr	
2278	GCC	TTT	CCT	GAT	AAG	CAG	AAT	GGC	ATC	GTT	
65▶	Ala	Phe	Pro	Asp	Lys	Gln	Asn	Gly	Ile	Val	
2308	CCG	GTG	GTG	GGC	GTT	GAT	GGC	TGG	TCC	CGC	
75▶	Pro	Val	Val	Gly	Val	Asp	Gly	Trp	Ser	Arg	
2338	ATC	ATC	AAT	GAA	AAC	CAG	CAG	TTT	GAT	GGC	
85▶	Ile	Ile	Asn	Glu	Asn	Gln	Gln	Phe	Asp	Gly	
2368	ATG	GAC	TTT	GAG	CAG	GAC	AAT	GAA	TCC	TGT	
95▶	Met	Asp	Phe	Glu	Gln	Asp	Asn	Glu	Ser	Cys	
2398	ACA	TGC	CGG	ATT	TAC	CGC	AAG	GAC	CGT	AAT	
105▶	Thr	Cys	Arg	Ile	Tyr	Arg	Lys	Asp	Arg	Asn	
2428	CAT	CCG	ATC	TGC	GTT	ACC	GAA	TGG	ATG	GAT	
115▶	His	Pro	Ile	Cys	Val	Thr	Glu	Trp	Met	Asp	
2458	GAA	TGC	CGC	CGC	GAA	CCA	TTC	AAA	ACT	CGC	
125▶	Glu	Cys	Arg	Arg	Glu	Pro	Phe	Lys	Thr	Arg	
2488	GAA	GGC	AGA	GAA	ATC	ACG	GGG	CCG	TGG	CAG	
135▶	Glu	Gly	Arg	Glu	Ile	Thr	Gly	Pro	Trp	Gln	

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Figure 14b (cont'd)

2518	TCG	CAT	CCC	AAA	CGG	ATG	TTA	CGT	CAT	AAA
145▶	Ser	His	Pro	Lys	Arg	Met	Leu	Arg	His	Lys
2548	GCC	ATG	ATT	CAG	TGT	GCC	CGT	CTG	GCC	TTC
155▶	Ala	Met	Ile	Gln	Cys	Ala	Arg	Leu	Ala	Phe
2578	GGA	TTT	GCT	GGT	ATC	TAT	GAC	AAG	GAT	GAA
165▶	Gly	Phe	Ala	Gly	Ile	Tyr	Asp	Lys	Asp	Glu
2608	GCC	GAG	CGC	ATT	GTC	GAA	AAT	ACT	GCA	TAC
175▶	Ala	Glu	Arg	Ile	Val	Glu	Asn	Thr	Ala	Tyr
	PstI									
2638	ACT	GCA	GAA	CGT	CAG	CCG	GAA	CGC	GAC	ATC
185▶	Thr	Ala	Glu	Arg	Gln	Pro	Glu	Arg	Asp	Ile
2668	ACT	CCG	GTT	AAC	GAT	GAA	ACC	ATG	CAG	GAG
195▶	Thr	Pro	Val	Asn	Asp	Glu	Thr	Met	Gln	Glu
2698	ATT	AAC	ACT	CTG	CTG	ATC	GCC	CTG	GAT	AAA
205▶	Ile	Asn	Thr	Leu	Leu	Ile	Ala	Leu	Asp	Lys
2728	ACA	TGG	GAT	GAC	GAC	TTA	TTG	CCG	CTC	TGT
215▶	Thr	Trp	Asp	Asp	Asp	Leu	Leu	Pro	Leu	Cys
2758	TCC	CAG	ATA	TTT	CGC	CGC	GAC	ATT	CGT	GCA
225▶	Ser	Gln	Ile	Phe	Arg	Arg	Asp	Ile	Arg	Ala
2788	TCG	TCA	GAA	CTG	ACA	CAG	GCC	GAA	GCA	GTA
235▶	Ser	Ser	Glu	Leu	Thr	Gln	Ala	Glu	Ala	Val
2818	AAA	GCT	CTT	GGA	TTC	CTG	AAA	CAG	AAA	GCC
245▶	Lys	Ala	Leu	Gly	Phe	Leu	Lys	Gln	Lys	Ala
	BglII XhoI									
2848	GCA	GAG	CAG	AAG	GTG	GCA	GCA	TAGATCTCGAG		
255▶	Ala	Glu	Gln	Lys	Val	Ala	Ala	•••		

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Figure 14b (cont'd)

HindIII

2880 AAGCTTCCTGCTGAACATCAAAGGCAAGAAAACATCTGT
 2919 TGTCAAAGACAGCATCCTTGAACAAGGACAATTAACAGT
 2958 TAACAAATAAAAACGCAAAAGAAAATGCCGATATCCTAT
 2997 TGGCATTTTTCTTTTATTTCTTATCAACATAAAGGTGAAT

XhoI

3036 CCCATACCTCGAGCTTCACGCTGCCGCAAGCACTCAGGG
 3075 CGCAAGGGCTGCTAAAAGGAAGCGGAACACGTAGAAAGC
 3114 CAGTCCGCAGAAACGGTGCTGACCCCGGATGAATGTCAG
 3153 CTACTGGGCTATCTGGACAAGGGAAAACGCAAGCGCAAA
 3192 GAGAAAGCAGGTAGCTTGCAGTGGGCTTACATGGCGATA
 3231 GCTAGACTGGGCGGTTTTATGGACAGCAAGCGAACCGGA

PvuII

3270 ATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCC
 3309 CTGCAAAGTAAACTGGATGGCTTTCTTGCCGCCAAGGAT

BglII

3348 CTGATGGCGCAGGGGATCAAGATCTGATCAAGAGACAGG
 3387 ATGAGGATCGTTTCGC ATG GAT ATT AAT ACT

1►Met Asp Ile Asn Thr

3418 GAA ACT GAG ATC AAG CAA AAG CAT TCA CTA
 6►Glu Thr Glu Ile Lys Gln Lys His Ser Leu

3448 ACC CCC TTT CCT GTT TTC CTA ATC AGC CCG
 16►Thr Pro Phe Pro Val Phe Leu Ile Ser Pro

3478 GCA TTT CGC GGG CGA TAT TTT CAC AGC TAT
 26►Ala Phe Arg Gly Arg Tyr Phe His Ser Tyr

3508 TTC AGG AGT TCA GCC ATG AAC GCT TAT TAC
 36►Phe Arg Ser Ser Ala Met Asn Ala Tyr Tyr

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Figure 14b (cont'd)

3538 ATT CAG GAT CGT CTT GAG GCT CAG AGC TGG
 46► Ile Gln Asp Arg Leu Glu Ala Gln Ser Trp
 3568 GCG CGT CAC TAC CAG CAG CTC GCC CGT GAA
 56► Ala Arg His Tyr Gln Gln Leu Ala Arg Glu
 3598 GAG AAA GAG GCA GAA CTG GCA GAC GAC ATG
 66► Glu Lys Glu Ala Glu Leu Ala Asp Asp Met
 3628 GAA AAA GGC CTG CCC CAG CAC CTG TTT GAA
 76► Glu Lys Gly Leu Pro Gln His Leu Phe Glu
 3658 TCG CTA TGC ATC GAT CAT TTG CAA CGC CAC
 86► Ser Leu Cys Ile Asp His Leu Gln Arg His
 3688 GGG GCC AGC AAA AAA TCC ATT ACC CGT GCG
 96► Gly Ala Ser Lys Lys Ser Ile Thr Arg Ala
 3718 TTT GAT GAC GAT GTT GAG TTT CAG GAG CGC
 106► Phe Asp Asp Asp Val Glu Phe Gln Glu Arg
 3748 ATG GCA GAA CAC ATC CGG TAC ATG GTT GAA
 116► Met Ala Glu His Ile Arg Tyr Met Val Glu
 3778 ACC ATT GCT CAC CAC CAG GTT GAT ATT GAT
 126► Thr Ile Ala His His Gln Val Asp Ile Asp
 HindIII
 3808 TCA GAG GTA TAA AACGAGTAGA AGC TTG GCT
 136► Ser Glu Val ...
 3839 GTT TTG GCG GAT GAG AGA AGA TTT TCA GCC
 3869 TGA TACAGATTAAATCAGAACGCAGAAAGCGGTCTGATA
 3907 AAACAGAATTTGCCTGGCGGCAGTAGCGCGGTGGTCCCA
 3946 CCTGACCCCATGCCGAACCTCAGAAGTGAAACGCCGTAGC
 3985 GCCGATGGTAGTGTGGGGTCTCCCCATGCGAGAGTAGGG

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Figure 14b (cont'd)

4024 AACTGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAA
4063 AGACTGGGCCTTTTCGTTTTATCTGTTGTTTGTCTGGTGAA
4102 CGCTCTCCTGAGTAGGACAAATCCGCCGGGAGCGGATTT
4141 GAACGTTGCGAAGCAACGGCCCCGGAGGGTGGCGGGGCAGG
4180 ACGCCCGCCATAAACTGCCAGGCATCAAATTAAGCAGAA
4219 GGCCATCCTGACGGATGGCCTTTTTTGCGTTTCTACAAAC
4258 TCTTTTGTTTATTTTTTCTAAATACATTCAAATATGTATC
4297 CGCTCATGAGACAATAACCCTGATAAATGCTTCAATAAT
4336 ATTGAAAAAGGAAGAGT ATG AGT ATT CAA CAT
1►Met Ser Ile Gln His
4368 TTC CGT GTC GCC CTT ATT CCC TTT TTT GCG
6►Phe Arg Val Ala Leu Ile Pro Phe Phe Ala
4398 GCA TTT TGC CTT CCT GTT TTT GCT CAC CCA
16►Ala Phe Cys Leu Pro Val Phe Ala His Pro
4428 GAA ACG CTG GTG AAA GTA AAA GAT GCT GAA
26►Glu Thr Leu Val Lys Val Lys Asp Ala Glu
4458 GAT CAG TTG GGT GCA CGA GTG GGT TAC ATC
36►Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile
4488 GAA CTG GAT CTC AAC AGC GGT AAG ATC CTT
46►Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu
4518 GAG AGT TTT CGC CCC GAA GAA CGT TTT CCA
56►Glu Ser Phe Arg Pro Glu Glu Arg Phe Pro
4548 ATG ATG AGC ACT TTT AAA GTT CTG CTA TGT
66►Met Met Ser Thr Phe Lys Val Leu Leu Cys

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Figure 14b (cont'd)

4578	GGC	GCG	GTA	TTA	TCC	CGT	GTT	GAC	GCC	GGG
76▶	Gly	Ala	Val	Leu	Ser	Arg	Val	Asp	Ala	Gly
4608	CAA	GAG	CAA	CTC	GGT	CGC	CGC	ATA	CAC	TAT
86▶	Gln	Glu	Gln	Leu	Gly	Arg	Arg	Ile	His	Tyr
							Scal			
4638	TCT	CAG	AAT	GAC	TTG	GTT	GAG	TAC	TCA	CCA
96▶	Ser	Gln	Asn	Asp	Leu	Val	Glu	Tyr	Ser	Pro
4668	GTC	ACA	GAA	AAG	CAT	CTT	ACG	GAT	GGC	ATG
106▶	Val	Thr	Glu	Lys	His	Leu	Thr	Asp	Gly	Met
4698	ACA	GTA	AGA	GAA	TTA	TGC	AGT	GCT	GCC	ATA
116▶	Thr	Val	Arg	Glu	Leu	Cys	Ser	Ala	Ala	Ile
4728	ACC	ATG	AGT	GAT	AAC	ACT	GCG	GCC	AAC	TTA
126▶	Thr	Met	Ser	Asp	Asn	Thr	Ala	Ala	Asn	Leu
4758	CTT	CTG	ACA	ACG	ATC	GGA	GGA	CCG	AAG	GAG
136▶	Leu	Leu	Thr	Thr	Ile	Gly	Gly	Pro	Lys	Glu
4788	CTA	ACC	GCT	TTT	TTG	CAC	AAC	ATG	GGG	GAT
146▶	Leu	Thr	Ala	Phe	Leu	His	Asn	Met	Gly	Asp
4818	CAT	GTA	ACT	CGC	CTT	GAT	CGT	TGG	GAA	CCG
156▶	His	Val	Thr	Arg	Leu	Asp	Arg	Trp	Glu	Pro
4848	GAG	CTG	AAT	GAA	GCC	ATA	CCA	AAC	GAC	GAG
166▶	Glu	Leu	Asn	Glu	Ala	Ile	Pro	Asn	Asp	Glu
4878	CGT	GAC	ACC	ACG	ATG	CCT	GTA	GCA	ATG	GCA
176▶	Arg	Asp	Thr	Thr	Met	Pro	Val	Ala	Met	Ala
4908	ACA	ACG	TTG	CGC	AAA	CTA	TTA	ACT	GGC	GAA
186▶	Thr	Thr	Leu	Arg	Lys	Leu	Leu	Thr	Gly	Glu
4938	CTA	CTT	ACT	CTA	GCT	TCC	CGG	CAA	CAA	TTA
196▶	Leu	Leu	Thr	Leu	Ala	Ser	Arg	Gln	Gln	Leu

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Figure 14b (cont'd)

4968 ATA GAC TGG ATG GAG GCG GAT AAA GTT GCA
206► Ile Asp Trp Met Glu Ala Asp Lys Val Ala

4998 GGA CCA CTT CTG CGC TCG GCC CTT CCG GCT
216► Gly Pro Leu Leu Arg Ser Ala Leu Pro Ala

5028 GGC TGG TTT ATT GCT GAT AAA TCT GGA GCC
226► Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala

5058 GGT GAG CGT GGG TCT CGC GGT ATC ATT GCA
236► Gly Glu Arg Gly Ser Arg Gly Ile Ile Ala

5088 GCA CTG GGG CCA GAT GGT AAG CCC TCC CGT
246► Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg

5118 ATC GTA GTT ATC TAC ACG ACG GGG AGT CAG
256► Ile Val Val Ile Tyr Thr Thr Gly Ser Gln

5148 GCA ACT ATG GAT GAA CGA AAT AGA CAG ATC
266► Ala Thr Met Asp Glu Arg Asn Arg Gln Ile

5178 GCT GAG ATA GGT GCC TCA CTG ATT AAG CAT
276► Ala Glu Ile Gly Ala Ser Leu Ile Lys His

5208 TGG TAA CTGTCAGACCAAGTTTACTCATATATACTTT
286► Trp . . .

5245 AGATTGATTTACGCGCCCTGTAGCGGCGCATTAAGCGCG

5284 GCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTT

5323 GCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTTCTTCCCT

5362 TCCTTTCTCGCCACGTTTCGCCGGCTTTCCCCGTCAAGCT

5401 CTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCT

5440 TTACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGAT

5479 GGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTT

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Figure 14b (cont'd)

5518 CGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGA
5557 CTCTTGTTCCAAACTTGAACAACACTCAACCCTATCTCG
5596 GGCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCG
5635 GCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTT
5674 AACGCGAATTTTAACAAAATATTAACGTTTACAATTTAA
5713 AAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGAC
5752 CAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTC
5791 AGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCC
5830 TTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAA
5869 ACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGA
5908 GCTACCAACTCTTTTTTCCGAAGGTAACTGGCTTCAGCAG
5947 AGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTA
5986 GTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTAC
6025 ATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGC
6064 CAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAG
6103 ACGATAGTTACCGGATAAGGCGCAGCGGTCTGGGCTGAAC
6142 GGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGAC
6181 CTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGA
6220 AAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTA
6259 TCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAG
6298 GGAGCTTCCAGGGGGGAAACGCCTGGTATCTTTATAGTCC
6337 TGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTT

Figure 14b (cont'd)

6376 GTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGC
6415 CAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTG
6454 GCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGA
6493 TTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGA
6532 TACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTC
6571 AGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTT
6610 TCTCCTTACGCATCTGTGCGGTATTTTCACACCGCATAGG
6649 GTCATGGCTGCGCCCCGACACCCGCCAACACCCGCTGAC
6688 GCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTAC
6727 AGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAG
6766 AGGTTTTTCACCGTCATCACCGAAACGCGCGAGGCAGCAA
6805 GGAGATGGCGCCCAACAGTCCCCCGGCCACGGGGCCTGC
6844 CACCATACCCACGCCGAAACAAGCGCTCATGAGCCCGAA
6883 GTGGCGAGCCCGATCTTCCCCATCGGTGATGTGGCGGAT
6922 ATAGGCGCCAGCAACCGCACCTGTGGCGCCGGTGATGCC
6961 GGCCACGATGCGTCCGGCGTAGAGGATCTGCTCATGTTT
7000 GACAGCTTATC

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT:
 - (A) NAME: European Molecular Biology Laboratory
(EMBL)
 - (B) STREET: Meyerhofstrasse 1
 - (C) CITY: Heidelberg
 - (E) COUNTRY: DE
 - (F) POSTAL CODE (ZIP): D-69117
- (ii) TITLE OF INVENTION: Novel DNA Cloning Method
- (iii) NUMBER OF SEQUENCES: 14
- (iv) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
(EPO)
- (vi) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: EP 97121562.2
 - (B) FILING DATE: 05-DEC-1997
- (vi) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: EP 98118756.0
 - (B) FILING DATE: 05-OCT-1998

(2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6150 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: both
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: pBAD24-recET
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (B) LOCATION: complement (96..974)
 - (D) OTHER INFORMATION: /product= "araC"
- (ix) FEATURE:
 - (A) NAME/KEY: misc_feature
 - (B) LOCATION: 1320..2162
 - (D) OTHER INFORMATION: /product= "t-recE"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 2155..2972
- (D) OTHER INFORMATION: /product= "recT"

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 3493..4353
- (D) OTHER INFORMATION: /product= "bla"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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ATCGATGCAT AATGTGCCTG TCAAATGGAC GAAGCAGGGA TTCTGCAAAC CCTATGCTAC      60
TCCGTCAAGC CGTCAATTGT CTGATTCTGT ACCAATTATG ACAACTTGAC GGCTACATCA      120
TTCACCTTTT CTTACAACC GGCACGGAAC TCGCTCGGGC TGGCCCCGGT GCATTTTTTA      180
AATACCCGCG AGAAATAGAG TTGATCGTCA AAACCAACAT TGCACCGAC GGTGGCGATA      240
GGCATCCGGG TGGTGCTCAA AAGCAGCTTC GCCTGGCTGA TACGTTGGTC CTCGCGCCAG      300
CTTAAGACGC TAATCCCTAA CTGCTGGCGG AAAAGATGTG ACAGACGCGA CGGCGACAAG      360
CAAACATGCT GTGCGACGCT GGCATATCA AAATTGCTGT CTGCCAGGTG ATCGCTGATG      420
TACTGACAAG CCTCGCGTAC CCGATTATCC ATCGGTGGAT GGAGCGACTC GTTAATCGCT      480
TCCATGCGCC GCAGTAACAA TTGCTCAAGC AGATTTATCG CCAGCAGCTC CGAATAGCGC      540
CCTTCCCCTT GCCCGGCGTT AATGATTTGC CCAAACAGGT CGCTGAAATG CGGCTGGTGC      600
GCTTCATCCG GCGGAAAGAA CCCCCTATTG GCAAATATTG ACGGCCAGTT AAGCCATTCA      660
TGCCAGTAGG CGCGCGGACG AAAGTAAACC CACTGGTGAT ACCATTGCGG AGCCTCCGGA      720
TGACGACCGT AGTGATGAAT CTCTCCTGGC GGGAACAGCA AAATATCACC CGGTCGGCAA      780
ACAAATTCTC GTCCCTGATT TTTCACCACC CCCTGACCGC GAATGGTGAG ATTGAGAATA      840
TAACCTTTCA TTCCAGCGG TCGGTCGATA AAAAAATCGA GATAACCGTT GGCCTCAATC      900
GGCGTTAAAC CCGCCACCAG ATGGGCATTA AACGAGTATC CCGGCAGCAG GGGATCATTT      960
TGCGCTTCAG CCATACTTTT CATACTCCCG CCATTGAGAG AAGAAACCAA TTGTCCATAT     1020
TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT TCTCGCTAAC CAAACCGGTA     1080
ACCCCGCTTA TAAAAGCAT TCTGTAACAA AGCGGGACCA AAGCCATGAC AAAACGCGT      1140
AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG ATTATTTGCA CGGCGTCACA     1200
CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG ATCCTACCTG ACGCTTTTTTA     1260
TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTTGGG CTAGCAGGAG GAATTCACCA     1320
TGGATCCCGT AATCGTAGAA GACATAGAGC CAGGTATTTA TTACGGAATT TCGAATGAGA     1380
ATTACCACGC GGGTCCCGGT ATCAGTAAGT CTCAGCTCGA TGACATTGCT GATACTCCGG      1440
CACTATATTT GTGGCGTAAA AATGCCCCCG TGGACACCAC AAAGACAAAA ACGCTCGATT      1500
TAGGAACTGC TTTCCACTGC CGGGTACTTG AACCGGAAGA ATTCAGTAAC CGCTTTATCG     1560
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TAGCACCTGA ATTTAACCGC CGTACAAACG CCGGAAAAGA AGAAGAGAAA GCGTTTCTGA 1620
TGGAATGCGC AAGCACAGGA AAAACGGTTA TCACTGCGGA AGAAGGCCGG AAAATTGAAC 1680
TCATGTATCA AAGCGTTATG GCTTTGCCGC TGGGGCAATG GCTTGTGAA AGCGCCGGAC 1740
ACGCTGAATC ATCAATTTAC TGGGAAGATC CTGAAACAGG AATTTTGTGT CCGTGCCGTC 1800
CGGACAAAAT TATCCCTGAA TTTCCTGGA TCATGGACGT GAAAACTACG GCGGATATTC 1860
AACGATTCAA AACCGCTTAT TACGACTACC GCTATCACGT TCAGGATGCA TTCTACAGTG 1920
ACGGTTATGA AGCACAGTTT GGAGTGACG CAACTTTCGT TTTTCTGGTT GCCAGCACAA 1980
CTATTGAATG CGGACGTTAT CCGGTTGAAA TTTTCATGAT GGGCGAAGAA GCAAACTGG 2040
CAGGTCAACA GGAATATCAC CGCAATCTGC GAACCCTGTC TGACTGCCTG AATACCGATG 2100
AATGGCCAGC TATTAAGACA TTATCACTGC CCCGCTGGGC TAAGGAATAT GCAAATGACT 2160
AAGCAACCAC CAATCGCAA AGCCGATCTG CAAAAAACTC AGGGAAACCG TGCACCAGCA 2220
GCAGTTAAAA ATAGCGACGT GATTAGTTTT ATTAACCAGC CATCAATGAA AGAGCAACTG 2280
GCAGCAGCTC TTCCACGCCA TATGACGGCT GAACGTATGA TCCGTATCGC CACCACAGAA 2340
ATTCTGTAAG TTCCGGCGTT AGGAACTGT GACACTATGA GTTTTGTGAG TGCATCGTA 2400
CAGTGTTCAC AGCTCGGACT TGAGCCAGGT AGCGCCCTCG GTCATGCATA TTTACTGCCT 2460
TTTGGAATA AAAACGAAAA GAGCGGTAAA AAGAACGTTT AGCTAATCAT TGGCTATCGC 2520
GGCATGATTG ATCTGGCTCG CCGTTCTGGT CAAATCGCCA GCCTGTCAGC CCGTGTGTC 2580
CGTGAAGGTG ACGAGTTTAG CTTCGAATTT GGCCTTGATG AAAAGTTAAT ACACCGCCCG 2640
GGAGAAAACG AAGATGCCCC GGTACCCAC GTCTATGCTG TCGCAAGACT GAAAGACGGA 2700
GGTACTCAGT TTGAAGTTAT GACGCGCAA CAGATTGAGC TGGTGCGCAG CCTGAGTAAA 2760
GCTGGTAATA ACGGGCCGTG GGTAACCTAC TGGGAAGAAA TGGCAAAGAA AACGGCTATT 2820
CGTCGCCTGT TCAATATTT GCCCGTATCA ATTGAGATCC AGCGTGCAGT ATCAATGGAT 2880
GAAAAGGAAC CACTGACAAT CGATCCTGCA GATTCCTCTG TATTAACCGG GGAATACAGT 2940
GTAATCGATA ATTCAGAGGA ATAGATCTAA GCTTGGCTGT TTTGGCGGAT GAGAGAAGAT 3000
TTTCAGCCTG ATACAGATTA AATCAGAACG CAGAAGCGGT CTGATAAAAC AGAATTTGCC 3060
TGGCGGCAGT AGCGCGGTGG TCCCACCTGA CCCCATGCCG AACTCAGAAG TGAAACGCCG 3120
TAGCGCCGAT GGTAGTGTGG GGTCTCCCCA TGCAGAGTA GGGAAGTGC AGGCATCAAA 3180
TAAAACGAAA GGCTCAGTCG AAAGACTGGG CCTTTCGTTT TATCTGTTGT TTGTCGGTGA 3240
ACGCTCTCCT GAGTAGGACA AATCCGCCGG GAGCGGATTT GAACGTTGCG AAGCAACGGC 3300
CCGGAGGGTG GCGGGCAGGA CGCCGCCAT AACTGCCAG GCATCAAATT AAGCAGAAGG 3360
CCATCCTGAC GGATGGCCTT TTTGCGTTTC TACAACTCT TTTGTTTATT TTTCTAAATA 3420
CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA 3480
AAAAGGAAGA GTATGAGTAT TCAACATTTT CGTGTGCCCC TTATTCCCTT TTTTGC GGCA 3540
TTTTGCCTTC CTGTTTTTGC TCACCCAGAA ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT 3600

CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG	3660
AGTTTTTCGCC CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC	3720
GCGGTATTAT CCCGTGTTGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT AACTATTCT	3780
CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA	3840
GTAAGAGAAT TATGCAGTGC TGCCATAACC ATGAGTGATA AACTGCGGC CAACTTACTT	3900
CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT	3960
GTAACTCGCC TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAA CGACGAGCGT	4020
GACACCACGA TGCCTGTAGC AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA	4080
CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG AGGCGGATAA AGTTGCAGGA	4140
CCACTTCTGC GCTCGGCCCT TCCGGCTGGC TGGTTTATTG CTGATAAATC TGGAGCCGGT	4200
GAGCGTGGGT CTCGCGGTAT CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC	4260
GTAGTTATCT ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT	4320
GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA CTCATATATA	4380
CTTTAGATTG ATTTACGCGC CCTGTAGCGG CGCATTAAGC GCGGCGGGTG TGGTGGTTAC	4440
GCGCAGCGTG ACCGCTACAC TTGCCAGCGC CCTAGCGCCC GCTCCTTTTCG CTTTCTTCCC	4500
TTCCTTTCTC GCCACGTTTC CCGGCTTTCC CCGTCAAGCT CTAAATCGGG GGCTCCCTTT	4560
AGGGTTCCGA TTTAGTGCTT TACGGCACCT CGACCCCAA AAACCTTGATT TGGGTGATGG	4620
TTCACGTAGT GGGCCATCGC CCTGATAGAC GGTTTTTCGC CTTTGACGT TGGAGTCCAC	4680
GTTCTTTAAT AGTGGACTCT TGTTCCAAAC TTGAACAACA CTCAACCCTA TCTCGGGCTA	4740
TTCTTTTGAT TTATAAGGGA TTTTGCCGAT TTCGGCCTAT TGGTTAAAAA ATGAGCTGAT	4800
TTAACAAAAA TTTAACGCGA ATTTTAACAA AATATTAACG TTTACAATTT AAAAGGATCT	4860
AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAATCCC TTAACGTGAG TTTTCGTTCC	4920
ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC	4980
GCGTAATCTG CTGCTTGCAA ACAAAAAAAC CACCGCTACC AGCGGTGGTT TGTTCGCCG	5040
ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA	5100
ATACTGTCCT TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGACCGC	5160
CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC GATAAGTCGT	5220
GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA	5280
CGGGGGGTTC GTGCACACAG CCCAGCTTGG AGCGAACGAC CTACACCGAA CTGAGATACC	5340
TACAGCGTGA GCTATGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC	5400
CGGTAAGCGG CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT	5460
GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA TTTTGTGAT	5520
GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC	5580
TGGCCTTTTG CTGGCCTTTT GCTCACATGT TCTTTCCTGC GTTATCCCCT GATTCTGTGG	5640

ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC 5700
 GCAGCGAGTC AGTGAGCGAG GAAGCGGAAG AGCGCCTGAT GCGGTATTTT CTCCTTACGC 5760
 ATCTGTGCGG TATTTACACAC CGCATAGGGT CATGGCTGCG CCCCACACC CGCCAACACC 5820
 CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC GCTTACAGAC AAGCTGTGAC 5880
 CGTCTCCGGG AGCTGCATGT GTCAGAGGTT TTCACCGTCA TCACCGAAAC GCGCGAGGCA 5940
 GCAAGGAGAT GGCGCCCAAC AGTCCCCCGG CCACGGGGCC TGCCACCATA CCCACGCCGA 6000
 AACAAGCGCT CATGAGCCCG AAGTGCGGAG CCCGATCTTC CCCATCGGTG ATGTCGGCGA 6060
 TATAGGCGCC AGCAACCGCA CCTGTGGCGC CGGTGATGCC GGCCACGATG CGTCCGGCGT 6120
 AGAGGATCTG CTCATGTTTG ACAGCTTATC 6150

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 843 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:

- (B) CLONE: t-recE

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..843
- (D) OTHER INFORMATION: /product= "t-recE"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

ATG GAT CCC GTA ATC GTA GAA GAC ATA GAG CCA GGT ATT TAT TAC GGA 48
 Met Asp Pro Val Ile Val Glu Asp Ile Glu Pro Gly Ile Tyr Tyr Gly
 1 5 10 15

ATT TCG AAT GAG AAT TAC CAC GCG GGT CCC GGT ATC AGT AAG TCT CAG 96
 Ile Ser Asn Glu Asn Tyr His Ala Gly Pro Gly Ile Ser Lys Ser Gln
 20 25 30

CTC GAT GAC ATT GCT GAT ACT CCG GCA CTA TAT TTG TGG CGT AAA AAT 144
 Leu Asp Asp Ile Ala Asp Thr Pro Ala Leu Tyr Leu Trp Arg Lys Asn
 35 40 45

GCC CCC GTG GAC ACC ACA AAG ACA AAA ACG CTC GAT TTA GGA ACT GCT 192
 Ala Pro Val Asp Thr Thr Lys Thr Lys Thr Leu Asp Leu Gly Thr Ala
 50 55 60

TTC CAC TGC CGG GTA CTT GAA CCG GAA GAA TTC AGT AAC CGC TTT ATC 240
 Phe His Cys Arg Val Leu Glu Pro Glu Glu Phe Ser Asn Arg Phe Ile
 65 70 75 80

GTA GCA CCT GAA TTT AAC CGC CGT ACA AAC GCC GGA AAA GAA GAA GAG 288
 Val Ala Pro Glu Phe Asn Arg Arg Thr Asn Ala Gly Lys Glu Glu
 85 90 95

AAA GCG TTT CAG ATG GAA TGC GCA AGC ACA GGA AAA ACG GTT ATC ACT 336
 Lys Ala Phe Leu Met Glu Cys Ala Ser Thr Gly Lys Thr Val Ile Thr
 100 105 110

GCG GAA GAA GGC CGG AAA ATT GAA CTC ATG TAT CAA AGC GTT ATG GCT Ala Glu Glu Gly Arg Lys Ile Glu Leu Met Tyr Gln Ser Val Met Ala 115 120 125	384
TTG CCG CTG GGG CAA TGG CTT GTT GAA AGC GCC GGA CAC GCT GAA TCA Leu Pro Leu Gly Gln Trp Leu Val Glu Ser Ala Gly His Ala Glu Ser 130 135 140	432
TCA ATT TAC TGG GAA GAT CCT GAA ACA GGA ATT TTG TGT CGG TGC CGT Ser Ile Tyr Trp Glu Asp Pro Glu Thr Gly Ile Leu Cys Arg Cys Arg 145 150 155 160	480
CCG GAC AAA ATT ATC CCT GAA TTT CAC TGG ATC ATG GAC GTG AAA ACT Pro Asp Lys Ile Ile Pro Glu Phe His Trp Ile Met Asp Val Lys Thr 165 170 175	528
ACG GCG GAT ATT CAA CGA TTC AAA ACC GCT TAT TAC GAC TAC CGC TAT Thr Ala Asp Ile Gln Arg Phe Lys Thr Ala Tyr Tyr Asp Tyr Arg Tyr 180 185 190	576
CAC GTT CAG GAT GCA TTC TAC AGT GAC GGT TAT GAA GCA CAG TTT GGA His Val Gln Asp Ala Phe Tyr Ser Asp Gly Tyr Glu Ala Gln Phe Gly 195 200 205	624
GTG CAG CCA ACT TTC GTT TTT CTG GTT GCC AGC ACA ACT ATT GAA TGC Val Gln Pro Thr Phe Val Phe Leu Val Ala Ser Thr Thr Ile Glu Cys 210 215 220	672
GGA CGT TAT CCG GTT GAA ATT TTC ATG ATG GGC GAA GAA GCA AAA CTG Gly Arg Tyr Pro Val Glu Ile Phe Met Met Gly Glu Glu Ala Lys Leu 225 230 235 240	720
GCA GGT CAA CAG GAA TAT CAC CGC AAT CTG CGA ACC CTG TCT GAC TGC Ala Gly Gln Gln Glu Tyr His Arg Asn Leu Arg Thr Leu Ser Asp Cys 245 250 255	768
CTG AAT ACC GAT GAA TGG CCA GCT ATT AAG ACA TTA TCA CTG CCC CGC Leu Asn Thr Asp Glu Trp Pro Ala Ile Lys Thr Leu Ser Leu Pro Arg 260 265 270	816
TGG GCT AAG GAA TAT GCA AAT GAC TAA Trp Ala Lys Glu Tyr Ala Asn Asp *	843
275 280	

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 281 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Asp Pro Val Ile Val Glu Asp Ile Glu Pro Gly Ile Tyr Tyr Gly 1 5 10 15
Ile Ser Asn Glu Asn Tyr His Ala Gly Pro Gly Ile Ser Lys Ser Gln 20 25 30
Leu Asp Asp Ile Ala Asp Thr Pro Ala Leu Tyr Leu Trp Arg Lys Asn 35 40 45
Ala Pro Val Asp Thr Thr Lys Thr Lys Thr Leu Asp Leu Gly Thr Ala 50 55 60

```

Phe His Cys Arg Val Leu Glu Pro Glu Glu Phe Ser Asn Arg Phe Ile
 65                               70                               75                               80
Val Ala Pro Glu Phe Asn Arg Arg Thr Asn Ala Gly Lys Glu Glu Glu
                               85                               90                               95
Lys Ala Phe Leu Met Glu Cys Ala Ser Thr Gly Lys Thr Val Ile Thr
 100                               105                               110
Ala Glu Glu Gly Arg Lys Ile Glu Leu Met Tyr Gln Ser Val Met Ala
 115                               120                               125
Leu Pro Leu Gly Gln Trp Leu Val Glu Ser Ala Gly His Ala Glu Ser
 130                               135                               140
Ser Ile Tyr Trp Glu Asp Pro Glu Thr Gly Ile Leu Cys Arg Cys Arg
 145                               150                               155                               160
Pro Asp Lys Ile Ile Pro Glu Phe His Trp Ile Met Asp Val Lys Thr
                               165                               170                               175
Thr Ala Asp Ile Gln Arg Phe Lys Thr Ala Tyr Tyr Asp Tyr Arg Tyr
 180                               185                               190
His Val Gln Asp Ala Phe Tyr Ser Asp Gly Tyr Glu Ala Gln Phe Gly
 195                               200                               205
Val Gln Pro Thr Phe Val Phe Leu Val Ala Ser Thr Thr Ile Glu Cys
 210                               215                               220
Gly Arg Tyr Pro Val Glu Ile Phe Met Met Gly Glu Glu Ala Lys Leu
 225                               230                               235
Ala Gly Gln Gln Glu Tyr His Arg Asn Leu Arg Thr Leu Ser Asp Cys
                               245                               250                               255
Leu Asn Thr Asp Glu Trp Pro Ala Ile Lys Thr Leu Ser Leu Pro Arg
                               260                               265                               270
Trp Ala Lys Glu Tyr Ala Asn Asp *
 275                               280

```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 810 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:

- (B) CLONE: rect

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..810
- (D) OTHER INFORMATION: /product= "rect"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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ATG ACT AAG CAA CCA CCA ATC GCA AAA GCC GAT CTG CAA AAA ACT CAG
Met Thr Lys Gln Pro Pro Ile Ala Lys Ala Asp Leu Gln Lys Thr Gln
 285                               290                               295

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GGA AAC CGT GCA CCA GCA GCA GTT AAA AAT AGC GAC GTG ATT AGT TTT Gly Asn Arg Ala Pro Ala Ala Val Lys Asn Ser Asp Val Ile Ser Phe 300 305 310	96
ATT AAC CAG CCA TCA ATG AAA GAG CAA CTG GCA GCA GCT CTT CCA CGC Ile Asn Gln Pro Ser Met Lys Glu Gln Leu Ala Ala Ala Leu Pro Arg 315 320 325	144
CAT ATG ACG GCT GAA CGT ATG ATC CGT ATC GCC ACC ACA GAA ATT CGT His Met Thr Ala Glu Arg Met Ile Arg Ile Ala Thr Thr Glu Ile Arg 330 335 340 345	192
AAA GTT CCG GCG TTA GGA AAC TGT GAC ACT ATG AGT TTT GTC AGT GCG Lys Val Pro Ala Leu Gly Asn Cys Asp Thr Met Ser Phe Val Ser Ala 350 355 360	240
ATC GTA CAG TGT TCA CAG CTC GGA CTT GAG CCA GGT AGC GCC CTC GGT Ile Val Gln Cys Ser Gln Leu Gly Leu Glu Pro Gly Ser Ala Leu Gly 365 370 375	288
CAT GCA TAT TTA CTG CCT TTT GGT AAT AAA AAC GAA AAG AGC GGT AAA His Ala Tyr Leu Leu Pro Phe Gly Asn Lys Asn Glu Lys Ser Gly Lys 380 385 390	336
AAG AAC GTT CAG CTA ATC ATT GGC TAT CGC GGC ATG ATT GAT CTG GCT Lys Asn Val Gln Leu Ile Ile Gly Tyr Arg Gly Met Ile Asp Leu Ala 395 400 405	384
CGC CGT TCT GGT CAA ATC GCC AGC CTG TCA GCC CGT GTT GTC CGT GAA Arg Arg Ser Gly Gln Ile Ala Ser Leu Ser Ala Arg Val Val Arg Glu 410 415 420 425	432
GGT GAC GAG TTT AGC TTC GAA TTT GGC CTT GAT GAA AAG TTA ATA CAC Gly Asp Glu Phe Ser Phe Glu Phe Gly Leu Asp Glu Lys Leu Ile His 430 435 440	480
CGC CCG GGA GAA AAC GAA GAT GCC CCG GTT ACC CAC GTC TAT GCT GTC Arg Pro Gly Glu Asn Glu Asp Ala Pro Val Thr His Val Tyr Ala Val 445 450 455	528
GCA AGA CTG AAA GAC GGA GGT ACT CAG TTT GAA GTT ATG ACG CGC AAA Ala Arg Leu Lys Asp Gly Gly Thr Gln Phe Glu Val Met Thr Arg Lys 460 465 470	576
CAG ATT GAG CTG GTG CGC AGC CTG AGT AAA GCT GGT AAT AAC GGG CCG Gln Ile Glu Leu Val Arg Ser Leu Ser Lys Ala Gly Asn Asn Gly Pro 475 480 485	624
TGG GTA ACT CAC TGG GAA GAA ATG GCA AAG AAA ACG GCT ATT CGT CGC Trp Val Thr His Trp Glu Glu Met Ala Lys Lys Thr Ala Ile Arg Arg 490 495 500 505	672
CTG TTC AAA TAT TTG CCC GTA TCA ATT GAG ATC CAG CGT GCA GTA TCA Leu Phe Lys Tyr Leu Pro Val Ser Ile Glu Ile Gln Arg Ala Val Ser 510 515 520	720
ATG GAT GAA AAG GAA CCA CTG ACA ATC GAT CCT GCA GAT TCC TCT GTA Met Asp Glu Lys Glu Pro Leu Thr Ile Asp Pro Ala Asp Ser Ser Val 525 530 535	768
TTA ACC GGG GAA TAC AGT GTA ATC GAT AAT TCA GAG GAA TAG Leu Thr Gly Glu Tyr Ser Val Ile Asp Asn Ser Glu Glu * 540 545 550	810

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 270 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```

Met Thr Lys Gln Pro Pro Ile Ala Lys Ala Asp Leu Gln Lys Thr Gln
 1              5              10              15
Gly Asn Arg Ala Pro Ala Ala Val Lys Asn Ser Asp Val Ile Ser Phe
              20              25              30
Ile Asn Gln Pro Ser Met Lys Glu Gln Leu Ala Ala Ala Leu Pro Arg
      35              40              45
His Met Thr Ala Glu Arg Met Ile Arg Ile Ala Thr Thr Glu Ile Arg
      50              55              60
Lys Val Pro Ala Leu Gly Asn Cys Asp Thr Met Ser Phe Val Ser Ala
      65              70              75              80
Ile Val Gln Cys Ser Gln Leu Gly Leu Glu Pro Gly Ser Ala Leu Gly
              85              90              95
His Ala Tyr Leu Leu Pro Phe Gly Asn Lys Asn Glu Lys Ser Gly Lys
      100              105              110
Lys Asn Val Gln Leu Ile Ile Gly Tyr Arg Gly Met Ile Asp Leu Ala
      115              120              125
Arg Arg Ser Gly Gln Ile Ala Ser Leu Ser Ala Arg Val Val Arg Glu
      130              135              140
Gly Asp Glu Phe Ser Phe Glu Phe Gly Leu Asp Glu Lys Leu Ile His
      145              150              155              160
Arg Pro Gly Glu Asn Glu Asp Ala Pro Val Thr His Val Tyr Ala Val
              165              170              175
Ala Arg Leu Lys Asp Gly Gly Thr Gln Phe Glu Val Met Thr Arg Lys
      180              185              190
Gln Ile Glu Leu Val Arg Ser Leu Ser Lys Ala Gly Asn Asn Gly Pro
      195              200              205
Trp Val Thr His Trp Glu Glu Met Ala Lys Lys Thr Ala Ile Arg Arg
      210              215              220
Leu Phe Lys Tyr Leu Pro Val Ser Ile Glu Ile Gln Arg Ala Val Ser
      225              230              235              240
Met Asp Glu Lys Glu Pro Leu Thr Ile Asp Pro Ala Asp Ser Ser Val
              245              250              255
Leu Thr Gly Glu Tyr Ser Val Ile Asp Asn Ser Glu Glu *
      260              265              270

```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 876 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:

- (B) CLONE: araC

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: complement (1..876)
- (D) OTHER INFORMATION: /product= "araC"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```

TGACAACTTG ACGGCTACAT CATTCACTTT TTCTTCACAA CCGGCACGGA ACTCGCTCGG      60
GCTGGCCCCG GTGCATTTTT TAAATACCCG CGAGAAATAG AGTTGATCGT CAAAACCAAC      120
ATTGCGACCG ACGGTGGCGA TAGGCATCCG GGTGGTGCTC AAAAGCAGCT TCGCCTGGCT      180
GATACGTTGG TCCTCGCGCC AGCTTAAGAC GCTAATCCCT AACTGCTGGC GGAAAAGATG      240
TGACAGACGC GACGGCGACA AGCAAACATG CTGTGCGACG CTGGCGATAT CAAAATTGCT      300
GTCTGCCAGG TGATCGCTGA TGTACTGACA AGCCTCGCGT ACCCGATTAT CCATCGGTGG      360
ATGGAGCGAC TCGTTAATCG CTTCCATGCG CCGCAGTAAC AATTGCTCAA GCAGATTTAT      420
CGCCAGCAGC TCCGAATAGC GCCCTTCCCC TTGCCCGGCG TTAATGATTT GCCCAAACAG      480
GTCGCTGAAA TCGGGCTGGT GCGCTTCATC CGGGCGAAAG AACCCCGTAT TGGCAAATAT      540
TGACGGCCAG TTAAGCCATT CATGCCAGTA GGCGCGCGGA CGAAAGTAAA CCCACTGGTG      600
ATACCATTCTG CGAGCCTCCG GATGACGACC GTAGTGATGA ATCTCTCCTG GCGGGAACAG      660
CAAAATATCA CCCGGTCGGC AAACAAATTC TCGTCCCTGA TTTTTCACCA CCCCTGACC      720
GCGAATGGTG AGATTGAGAA TATAACCTTT CATTCCCAGC GGTCGGTCGA TAAAAAATC      780
GAGATAACCG TTGGCCTCAA TCGGCGTTAA ACCCGCCACC AGATGGGCAT TAAACGAGTA      840
TCCCGGCAGC AGGGGATCAT TTTGCGCTTC AGCCAT                                876

```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 292 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```

Met Ala Glu Ala Gln Asn Asp Pro Leu Leu Pro Gly Tyr Ser Phe Asn
 1             5             10             15
Ala His Leu Val Ala Gly Leu Thr Pro Ile Glu Ala Asn Gly Tyr Leu
          20             25             30

```

```

Asp Phe Phe Ile Asp Arg Pro Leu Gly Met Lys Gly Tyr Ile Leu Asn
   35                               40                               45
Leu Thr Ile Arg Gly Gln Gly Val Val Lys Asn Gln Gly Arg Glu Phe
   50                               55                               60
Val Cys Arg Pro Gly Asp Ile Leu Leu Phe Pro Pro Gly Glu Ile His
   65                               70                               75                               80
His Tyr Gly Arg His Pro Glu Ala Arg Glu Trp Tyr His Gln Trp Val
                               85                               90                               95
Tyr Phe Arg Pro Arg Ala Tyr Trp His Glu Trp Leu Asn Trp Pro Ser
                               100                               105                               110
Ile Phe Ala Asn Thr Gly Phe Phe Arg Pro Asp Glu Ala His Gln Pro
   115                               120                               125
His Phe Ser Asp Leu Phe Gly Gln Ile Ile Asn Ala Gly Gln Gly Glu
   130                               135                               140
Gly Arg Tyr Ser Glu Leu Leu Ala Ile Asn Leu Leu Glu Gln Leu Leu
   145                               150                               155                               160
Leu Arg Arg Met Glu Ala Ile Asn Glu Ser Leu His Pro Pro Met Asp
                               165                               170                               175
Asn Arg Val Arg Glu Ala Cys Gln Tyr Ile Ser Asp His Leu Ala Asp
                               180                               185                               190
Ser Asn Phe Asp Ile Ala Ser Val Ala Gln His Val Cys Leu Ser Pro
   195                               200                               205
Ser Arg Leu Ser His Leu Phe Arg Gln Gln Leu Gly Ile Ser Val Leu
   210                               215                               220
Ser Trp Arg Glu Asp Gln Arg Ile Ser Gln Ala Lys Leu Leu Leu Ser
   225                               230                               235                               240
Thr Thr Arg Met Pro Ile Ala Thr Val Gly Arg Asn Val Gly Phe Asp
                               245                               250                               255
Asp Gln Leu Tyr Phe Ser Arg Val Phe Lys Lys Cys Thr Gly Ala Ser
   260                               265                               270
Pro Ser Glu Phe Arg Ala Gly Cys Glu Glu Lys Val Asn Asp Val Ala
   275                               280                               285
Val Lys Leu Ser
   290

```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 861 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:

- (B) CLONE: bla

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION:1..861

(D) OTHER INFORMATION:/product= "bla"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

ATG AGT ATT CAA CAT TTC CGT GTC GCC CTT ATT CCC TTT TTT GCG GCA	48
Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala	
295 300 305	
TTT TGC CTT CCT GTT TTT GCT CAC CCA GAA ACG CTG GTG AAA GTA AAA	96
Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu Val Lys Val Lys	
310 315 320	
GAT GCT GAA GAT CAG TTG GGT GCA CGA GTG GGT TAC ATC GAA CTG GAT	144
Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp	
325 330 335 340	
CTC AAC AGC GGT AAG ATC CTT GAG AGT TTT CGC CCC GAA GAA CGT TTT	192
Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg Phe	
345 350 355	
CCA ATG ATG AGC ACT TTT AAA GTT CTG CTA TGT GGC GCG GTA TTA TCC	240
Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser	
360 365 370	
CGT GTT GAC GCC GGG CAA GAG CAA CTC GGT CGC CGC ATA CAC TAT TCT	288
Arg Val Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser	
375 380 385	
CAG AAT GAC TTG GTT GAG TAC TCA CCA GTC ACA GAA AAG CAT CTT ACG	336
Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His Leu Thr	
390 395 400	
GAT GGC ATG ACA GTA AGA GAA TTA TGC AGT GCT GCC ATA ACC ATG AGT	384
Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser	
405 410 415 420	
GAT AAC ACT GCG GCC AAC TTA CTT CTG ACA ACG ATC GGA GGA CCG AAG	432
Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys	
425 430 435	
GAG CTA ACC GCT TTT TTG CAC AAC ATG GGG GAT CAT GTA ACT CGC CTT	480
Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His Val Thr Arg Leu	
440 445 450	
GAT CGT TGG GAA CCG GAG CTG AAT GAA GCC ATA CCA AAC GAC GAG CGT	528
Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu Arg	
455 460 465	
GAC ACC ACG ATG CCT GTA GCA ATG GCA ACA ACG TTG CGC AAA CTA TTA	576
Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu Arg Lys Leu Leu	
470 475 480	
ACT GGC GAA CTA CTT ACT CTA GCT TCC CGG CAA CAA TTA ATA GAC TGG	624
Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp	
485 490 495 500	
ATG GAG GCG GAT AAA GTT GCA GGA CCA CTT CTG CGC TCG GCC CTT CCG	672
Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg Ser Ala Leu Pro	
505 510 515	
GCT GGC TGG TTT ATT GCT GAT AAA TCT GGA GCC GGT GAG CGT GGG TCT	720
Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly Ser	
520 525 530	

CGC GGT ATC ATT GCA GCA CTG GGG CCA GAT GGT AAG CCC TCC CGT ATC	768
Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile	
535 540 545	
GTA GTT ATC TAC ACG ACG GGG AGT CAG GCA ACT ATG GAT GAA CGA AAT	816
Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn	
550 555 560	
AGA CAG ATC GCT GAG ATA GGT GCC TCA CTG ATT AAG CAT TGG TAA	861
Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp *	
565 570 575	

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 287 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Met	Ser	Ile	Gln	His	Phe	Arg	Val	Ala	Leu	Ile	Pro	Phe	Phe	Ala	Ala	1	5	10	15
Phe	Cys	Leu	Pro	Val	Phe	Ala	His	Pro	Glu	Thr	Leu	Val	Lys	Val	Lys	20	25	30	
Asp	Ala	Glu	Asp	Gln	Leu	Gly	Ala	Arg	Val	Gly	Tyr	Ile	Glu	Leu	Asp	35	40	45	
Leu	Asn	Ser	Gly	Lys	Ile	Leu	Glu	Ser	Phe	Arg	Pro	Glu	Glu	Arg	Phe	50	55	60	
Pro	Met	Met	Ser	Thr	Phe	Lys	Val	Leu	Leu	Cys	Gly	Ala	Val	Leu	Ser	65	70	75	80
Arg	Val	Asp	Ala	Gly	Gln	Glu	Gln	Leu	Gly	Arg	Arg	Ile	His	Tyr	Ser	85	90	95	
Gln	Asn	Asp	Leu	Val	Glu	Tyr	Ser	Pro	Val	Thr	Glu	Lys	His	Leu	Thr	100	105	110	
Asp	Gly	Met	Thr	Val	Arg	Glu	Leu	Cys	Ser	Ala	Ala	Ile	Thr	Met	Ser	115	120	125	
Asp	Asn	Thr	Ala	Ala	Asn	Leu	Leu	Leu	Thr	Thr	Ile	Gly	Gly	Pro	Lys	130	135	140	
Glu	Leu	Thr	Ala	Phe	Leu	His	Asn	Met	Gly	Asp	His	Val	Thr	Arg	Leu	145	150	155	160
Asp	Arg	Trp	Glu	Pro	Glu	Leu	Asn	Glu	Ala	Ile	Pro	Asn	Asp	Glu	Arg	165	170	175	
Asp	Thr	Thr	Met	Pro	Val	Ala	Met	Ala	Thr	Thr	Leu	Arg	Lys	Leu	Leu	180	185	190	
Thr	Gly	Glu	Leu	Leu	Thr	Leu	Ala	Ser	Arg	Gln	Gln	Leu	Ile	Asp	Trp	195	200	205	
Met	Glu	Ala	Asp	Lys	Val	Ala	Gly	Pro	Leu	Leu	Arg	Ser	Ala	Leu	Pro	210	215	220	
Ala	Gly	Trp	Phe	Ile	Ala	Asp	Lys	Ser	Gly	Ala	Gly	Glu	Arg	Gly	Ser				

225		230		235		240
Arg Gly Ile Ile	Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile					
	245		250		255	
Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn						
	260		265		270	
Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp *						
	275		280		285	

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7195 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:

- (B) CLONE: pBAD-ETgamma

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 3588..4004
- (D) OTHER INFORMATION: /product= "red gamma"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

ATCGATGCAT AATGTGCCTG TCAAATGGAC GAAGCAGGGA TTCTGCAAAC CCTATGCTAC	60
TCCGTCAAGC CGTCAATTGT CTGATTCGTT ACCAATTATG ACAACTTGAC GGCTACATCA	120
TTCACTTTTT CTTCAACAACC GGCACGGAAC TCGCTCGGGC TGGCCCCGGT GCATTTTTTTA	180
AATACCCGCG AGAAATAGAG TTGATCGTCA AAACCAACAT TGCGACCGAC GGTGGCGATA	240
GGCATCCGGG TGGTGCTCAA AAGCAGCTTC GCCTGGCTGA TACGTTGGTC CTCGCGCCAG	300
CTTAAGACGC TAATCCCTAA CTGCTGGCGG AAAAGATGTG ACAGACGCGA CGGCGACAAG	360
CAAACATGCT GTGCGACGCT GGCATATCA AAATTGCTGT CTGCCAGGTG ATCGCTGATG	420
TACTGACAAG CCTCGGTAC CCGATTATCC ATCGGTGGAT GGAGCGACTC GTTAATCGCT	480
TCCATGCGCC GCAGTAACAA TTGCTCAAGC AGATTTATCG CCAGCAGCTC CGAATAGCGC	540
CCTTCCCCTT GCCCGGCGTT AATGATTTGC CCAAACAGGT CGCTGAAATG CGGCTGGTGC	600
GCTTCATCCG GGCGAAAGAA CCCCCTATTG GCAAATATTG ACGGCCAGTT AAGCCATTCA	660
TGCCAGTAGG CGCGCGGACG AAAGTAAACC CACTGGTGAT ACCATTCGCG AGCCTCCGGA	720
TGACGACCGT AGTGATGAAT CTCTCCTGGC GGGAACAGCA AAATATCACC CGGTGCGCAA	780
ACAAATTCTC GTCCCTGATT TTTCACCACC CCCTGACCGC GAATGGTGAG ATTGAGAATA	840
TAACCTTTCA TTCCAGCGG TCGGTCGATA AAAAAATCGA GATAACCGTT GGCCTCAATC	900
GGCGTTAAAC CCGCCACCAG ATGGGCATTA AACGAGTATC CCGGCAGCAG GGGATCATTT	960
TGCGCTTCAG CCATACTTTT CATACTCCCG CCATTTCAGAG AAGAAACCAA TTGTCCATAT	1020

TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT TCTCGCTAAC CAAACCGGTA 1080
ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA AAGCCATGAC AAAAACGCGT 1140
AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG ATTATTTGCA CGGCGTCACA 1200
CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG ATCCTACCTG ACGCTTTTTA 1260
TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTTGGG CTAGCAGGAG GAATTCACCA 1320
TGGATCCCGT AATCGTAGAA GACATAGAGC CAGGTATTTA TTACGGAATT TCGAATGAGA 1380
ATTACCACGC GGGTCCCGT ATCAGTAAGT CTCAGCTCGA TGACATTGCT GATACTCCGG 1440
CACTATATTT GTGGCGTAAA AATGCCCCCG TGGACACCAC AAAGACAAAA ACGCTCGATT 1500
TAGGAACTGC TTTCCACTGC CGGGTACTTG AACCAGGAAGA ATTCAGTAAC CGCTTTATCG 1560
TAGCACCTGA ATTTAACCGC CGTACAAACG CCGGAAAAGA AGAAGAGAAA GCGTTTCTGA 1620
TGGAATGCGC AAGCACAGGA AAAACGGTTA TCACTGCGGA AGAAGGCCGG AAAATTGAAC 1680
TCATGTATCA AAGCGTTATG GCTTTGCCGC TGGGGCAATG GCTTGTGAA AGCGCCGGAC 1740
ACGCTGAATC ATCAATTTAC TGGGAAGATC CTGAAACAGG AATTTTGTGT CGGTGCCGTC 1800
CGGACAAAAT TATCCCTGAA TTTCACTGGA TCATGGACGT GAAAACCTACG GCGGATATTC 1860
AACGATTCAA AACCCTTAT TACGACTACC GCTATCACGT TCAGGATGCA TTCTACAGTG 1920
ACGGTTATGA AGCACAGTTT GGAGTGCAGC CAACTTTCGT TTTCTGGTT GCCAGCACAA 1980
CTATTGAATG CGGACGTTAT CCGGTTGAAA TTTTCATGAT GGGCGAAGAA GCAAACTGG 2040
CAGGTCAACA GGAATATCAC CGCAATCTGC GAACCCTGTC TGACTGCCTG AATACCGATG 2100
AATGGCCAGC TATTAAGACA TTATCACTGC CCCGCTGGGC TAAGGAATAT GCAAATGACT 2160
AGATCTCGAG GTACCCGAGC ACGTGTGAC AATTAATCAT CGGCATAGTA TATCGGCATA 2220
GTATAATACG ACAAGGTGAG GAACTAAACC ATGGCTAAGC AACCACCAAT CGCAAAGCC 2280
GATCTGCAAA AAACCTCAGG AAACCGTGCA CCAGCAGCAG TTAAAAATAG CGACGTGATT 2340
AGTTTTATTA ACCAGCCATC AATGAAAGAG CAACTGGCAG CAGCTCTTCC ACGCCATATG 2400
ACGGCTGAAC GTATGATCCG TATCGCCACC ACAGAAATTC GTAAAGTTCC GCGGTTAGGA 2460
AACTGTGACA CTATGAGTTT TGTCAGTGCG ATCGTACAGT GTTCACAGCT CGGACTTGAG 2520
CCAGGTAGCG CCCTCGGTCA TGCATATTTA CTGCCTTTTG GTAATAAAAA CGAAAAGAGC 2580
GGTAAAAAGA ACGTTCAGCT AATCATTTGC TATCGCGGCA TGATTGATCT GGCTCGCCGT 2640
TCTGGTCAAA TCGCCAGCCT GTCAGCCCGT GTTGTCCTG AAGGTGACGA GTTTAGCTTC 2700
GAATTTGGCC TTGATGAAAA GTTAATACAC CGCCCGGGAG AAAACGAAGA TGCCCCGGTT 2760
ACCCACGTCT ATGCTGTGCG AAGACTGAAA GACGGAGGTA CTCAGTTTGA AGTTATGACG 2820
CGCAAACAGA TTGAGCTGGT GCGCAGCCTG AGTAAAGCTG GTAATAACGG GCCGTGGGTA 2880
ACTCACTGGG AAGAAATGGC AAAGAAAACG GCTATTCGTC GCCTGTTCAA ATATTTGCCC 2940
GTATCAATTG AGATCCAGCG TGCAGTATCA ATGGATGAAA AGGAACCACT GACAATCGAT 3000
CCTGCAGATT CCTCTGTATT AACCAGGGGAA TACAGTGTA TCGATAATTC AGAGGAATAG 3060

ATCTAAGCTT CCTGCTGAAC ATCAAAGGCA AGAAAACATC TGTGTGCAAA GACAGCATCC 3120
TTGAACAAGG ACAATTAACA GTTAACAAAT AAAACGCAA AAGAAAATGC CGATATCCTA 3180
TTGGCATTTC CTTTTATTTC TTATCAACAT AAAGGTGAAT CCCATACCTC GAGCTTCACG 3240
CTGCCGCAAG CACTCAGGGC GCAAGGGCTG CTAAAAGGAA GCGGAACACG TAGAAAGCCA 3300
GTCCGCAGAA ACGGTGCTGA CCCC GGATGA ATGTCAGCTA CTGGGCTATC TGGACAAGGG 3360
AAAACGCAAG CGCAAAGAGA AAGCAGGTAG CTTGCAGTGG GCTTACATGG CGATAGCTAG 3420
ACTGGGCGGT TTTATGGACA GCAAGCGAAC CGGAATTGCC AGCTGGGGCG CCCTCTGGTA 3480
AGGTTGGGAA GCCCTGCAA GTAAACTGGA TGGCTTTCTT GCCGCCAAGG ATCTGATGGC 3540
GCAGGGGATC AAGATCTGAT CAAGAGACAG GATGAGGATC GTTTCGCATG GATATTAATA 3600
CTGAAACTGA GATCAAGCAA AAGCATTCAC TAACCCCTT TCCTGTTTTT CTAATCAGCC 3660
CGGCATTTTC CGGGCGATAT TTTCACAGCT ATTT CAGGAG TTCAGCCATG AACGCTTATT 3720
ACATTCAGGA TCGTCTTGAG GCTCAGAGCT GGGCGCGTCA CTACCAGCAG CTCGCCCCGTG 3780
AAGAGAAAGA GGCAGAACTG GCAGACGACA TGGAAAAGG CCTGCCCCAG CACCTGTTTG 3840
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CGTTTGATGA CGATGTTGAG TTTCAGGAGC GCATGGCAGA ACACATCCGG TACATGGTTG 3960
AAACCATTGC TCACCACCAG GTTGATATTG ATTCAGAGGT ATAAAACGAG TAGAAGCTTG 4020
GCTGTTTTTG CGGATGAGAG AAGATTTTCA GCCTGATACA GATTAAATCA GAACGCAGAA 4080
GCGGTCTGAT AAAACAGAA TTGCCTGGCG GCAGTAGCGC GGTGGTCCCA CCTGACCCCA 4140
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GAGTAGGGAA CTGCCAGGCA TCAAATAAAA CGAAAGGCTC AGTCGAAAGA CTGGGCCTTT 4260
CGTTTTATCT GTTGTGTTGTC GGTGAACGCT CTCCTGAGTA GGACAAATCC GCCGGGAGCG 4320
GATTTGAACG TTGCGAAGCA ACGGCCCGGA GGGTGGCGGG CAGGACGCCC GCCATAAACT 4380
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GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTAC TCGAACTGGA 4680
TCTCAACAGC GGTAAGATCC TTGAGAGTT TCGCCCCGAA GAACGTTTTT CAATGATGAG 4740
CACTTTTAAA GTTCTGCTAT GTGGCGCGGT ATTATCCCGT GTTGACGCCG GGCAAGAGCA 4800
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AAAGCATCTT ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG 4920
TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG AGCTAACCGC 4980
TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA 5040
TGAAGCCATA CCAAACGACG AGCGTGACAC CACGATGCCT GTAGCAATGG CAACAACGTT 5100

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GATGGAGGCG GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT 5220
TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG CAGCACTGGG 5280
GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT 5340
GGATGAACGA AATAGACAGA TCGCTGAGAT AGGTGCCTCA CTGATTAAGC ATTGGTAACT 5400
GTCAGACCAA GTTTACTCAT ATATACTTTA GATTGATTTA CGCGCCCTGT AGCGGCGCAT 5460
TAAGCGCGGC GGGTGTGGTG GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG 5520
CGCCCGCTCC TTTGCTTTT TCCCTTCTT TTCTCGCCAC GTTCGCCGGC TTTCCCGTCC 5580
AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTAG TGCTTTACGG CACCTCGACC 5640
CCAAAAAAGT TGATTTGGGT GATGGTTTAC GTAGTGGGCC ATCGCCCTGA TAGACGGTTT 5700
TTCGCCCTTT GACGTTGGAG TCCACGTTCT TTAATAGTGG ACTCTTGTT CAAACTTGAA 5760
CAACACTCAA CCTATCTCG GGCTATTCTT TTGATTTATA AGGGATTTTG CCGATTTCCG 5820
CCTATTGGTT AAAAAATGAG CTGATTTAAC AAAAAATTAA CGCGAATTTT AACAAAATAT 5880
TAACGTTTAC AATTTAAAAAG GATCTAGGTG AAGATCCTTT TTGATAATCT CATGACCAA 5940
ATCCCTTAAC GTGAGTTTTC GTTCCACTGA GCGTCAGACC CCGTAGAAAA GATCAAAGGA 6000
TCTTCTTGAG ATCCTTTTTT TCTGCGCGTA ATCTGCTGCT TGCAAACAAA AAAACCACCG 6060
CTACCAGCGG TGTTTGTGTT GCCGGATCAA GAGCTACCAA CTCTTTTTTC GAAGGTAAGT 6120
GGCTTCAGCA GAGCGCAGAT ACCAAATACT GTCCTTCTAG TGTAGCCGTA GTTAGGCCAC 6180
CACTTCAAGA ACTCTGTAGC ACCGCCTACA TACCTCGCTC TGCTAATCCT GTTACCAGTG 6240
GCTGCTGCCA GTGGCGATAA GTCGTGTCTT ACCGGGTGG ACTCAAGACG ATAGTTACCG 6300
GATAAGGCGC AGCGGTCGGG CTGAACGGGG GGTTCGTGCA CACAGCCAG CTTGGAGCGA 6360
ACGACCTACA CCGAACTGAG ATACCTACAG CGTGAGCTAT GAGAAAGCGC CACGCTTCCC 6420
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AGGGAGCTTC CAGGGGGAAA CGCCTGGTAT CTTTATAGTC CTGTGGGGTT TCGCCACCTC 6540
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AGCAACGCGG CCTTTTTACG GTTCCTGGCC TTTTGCTGGC CTTTGTCTCA CATGTTCTTT 6660
CCTGCGTTAT CCCCTGATTC TGTGGATAAC CGTATTACCG CCTTTGAGTG AGCTGATACC 6720
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CTGCGCCCCG ACACCCGCCA ACACCCGCTG ACGCGCCCTG ACGGGCTTGT CTGCTCCCCG 6900
CATCCGCTTA CAGACAAGCT GTGACCGTCT CCGGGAGCTG CATGTGTCAG AGGTTTTTAC 6960
CGTCATCACC GAAACGCGCG AGGCAGCAAG GAGATGGCGC CCAACAGTCC CCCGGCCACG 7020
GGGCTGCCA CCATACCCAC GCCGAAACAA GCGCTCATGA GCGGGAAGTG GCGAGCCCGA 7080
TCTTCCCAT CCGTGATGTC GGCGATATAG GCGCCAGCAA CCGCACCTGT GGCGCCGGTG 7140

ATGCCGGCCA CGATGCGTCC GGCCTAGAGG ATCTGCTCAT GTTGACAGC TTATC

7195

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7010 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: both

(vii) IMMEDIATE SOURCE:

- (B) CLONE: pBAD-alpha-beta-gamma

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:1320..2000
- (D) OTHER INFORMATION:/product= "red alpha"

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:2086..2871
- (D) OTHER INFORMATION:/product= "red beta"

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:3403..3819
- (D) OTHER INFORMATION:/product= "red gamma"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

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TTCAC TTTT CTTCAACAACC GGCACGGAAC TCGCTCGGGC TGGCCCCGGT GCATTTTTTTA	180
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GGCATCCGGG TGGTGCTCAA AAGCAGCTTC GCCTGGCTGA TACGTTGGTC CTCGCGCCAG	300
CTTAAGACGC TAATCCCTAA CTGCTGGCGG AAAAGATGTG ACAGACGCGA CGGCGACAAG	360
CAAACATGCT GTGCGACGCT GGCATATCA AAATTGCTGT CTGCCAGGTG ATCGCTGATG	420
TACTGACAAG CCTCGCGTAC CCGATTATCC ATCGGTGGAT GGAGCGACTC GTTAATCGCT	480
TCCATGCGCC GCAGTAACAA TTGCTCAAGC AGATTTATCG CCAGCAGCTC CGAATAGCGC	540
CCTTCCCCTT GCCCGGCGTT AATGATTTGC CCAAACAGGT CGTGAAATG CGGCTGGTGC	600
GCTTCATCCG GGCAGAAAGAA CCCCCTATTG GCAAATATTG ACGGCCAGTT AAGCCATTCA	660
TGCCAGTAGG CGCGCGGACG AAAGTAAACC CACTGGTGAT ACCATTGCGG AGCCTCCGGA	720
TGACGACCGT AGTGATGAAT CTCTCCTGGC GGGAACAGCA AAATATCACC CGGTGCGCAA	780
ACAAATTCTC GTCCCTGATT TTTCACCACC CCCTGACCGC GAATGGTGAG ATTGAGAATA	840
TAACCTTTCA TTCCCAGCGG TCGGTGCGATA AAAAAATCGA GATAACCGTT GGCCTCAATC	900
GGCGTTAAAC CCGCCACCAG ATGGGCATTA AACGAGTATC CCGGCAGCAG GGGATCATTT	960

TCGCGCTTCAG CCATACTTTT CATACTCCCG CCATTCAGAG AAGAAACCAA TTGTCCATAT 1020
 TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT TCTCGCTAAC CAAACCGGTA 1080
 ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA AAGCCATGAC AAAACGCGT 1140
 AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG ATTATTGCA CGGCGTCACA 1200
 CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG ATCCTACCTG ACGCTTTTTA 1260
 TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTGGG CTAGCAGGAG GAATTCACC 1319
 ATG ACA CCG GAC ATT ATC CTG CAG CGT ACC GGG ATC GAT GTG AGA GCT 1367
 Met Thr Pro Asp Ile Ile Leu Gln Arg Thr Gly Ile Asp Val Arg Ala
 290 295 300
 GTC GAA CAG GGG GAT GAT GCG TGG CAC AAA TTA CGG CTC GGC GTC ATC 1415
 Val Glu Gln Gly Asp Asp Ala Trp His Lys Leu Arg Leu Gly Val Ile
 305 310 315
 ACC GCT TCA GAA GTT CAC AAC GTG ATA GCA AAA CCC CGC TCC GGA AAG 1463
 Thr Ala Ser Glu Val His Asn Val Ile Ala Lys Pro Arg Ser Gly Lys
 320 325 330 335
 AAG TGG CCT GAC ATG AAA ATG TCC TAC TTC CAC ACC CTG CTT GCT GAG 1511
 Lys Trp Pro Asp Met Lys Met Ser Tyr Phe His Thr Leu Leu Ala Glu
 340 345 350
 GTT TGC ACC GGT GTG GCT CCG GAA GTT AAC GCT AAA GCA CTG GCC TGG 1559
 Val Cys Thr Gly Val Ala Pro Glu Val Asn Ala Lys Ala Leu Ala Trp
 355 360 365
 GGA AAA CAG TAC GAG AAC GAC GCC AGA ACC CTG TTT GAA TTC ACT TCC 1607
 Gly Lys Gln Tyr Glu Asn Asp Ala Arg Thr Leu Phe Glu Phe Thr Ser
 370 375 380
 GGC GTG AAT GTT ACT GAA TCC CCG ATC ATC TAT CGC GAC GAA AGT ATG 1655
 Gly Val Asn Val Thr Glu Ser Pro Ile Ile Tyr Arg Asp Glu Ser Met
 385 390 395
 CGT ACC GCC TGC TCT CCC GAT GGT TTA TGC AGT GAC GGC AAC GGC CTT 1703
 Arg Thr Ala Cys Ser Pro Asp Gly Leu Cys Ser Asp Gly Asn Gly Leu
 400 405 410 415
 GAA CTG AAA TGC CCG TTT ACC TCC CGG GAT TTC ATG AAG TTC CGG CTC 1751
 Glu Leu Lys Cys Pro Phe Thr Ser Arg Asp Phe Met Lys Phe Arg Leu
 420 425 430
 GGT GGT TTC GAG GCC ATA AAG TCA GCT TAC ATG GCC CAG GTG CAG TAC 1799
 Gly Gly Phe Glu Ala Ile Lys Ser Ala Tyr Met Ala Gln Val Gln Tyr
 435 440 445
 AGC ATG TGG GTG ACG CGA AAA AAT GCC TGG TAC TTT GCC AAC TAT GAC 1847
 Ser Met Trp Val Thr Arg Lys Asn Ala Trp Tyr Phe Ala Asn Tyr Asp
 450 455 460
 CCG CGT ATG AAG CGT GAA GGC CTG CAT TAT GTC GTG ATT GAG CGG GAT 1895
 Pro Arg Met Lys Arg Glu Gly Leu His Tyr Val Val Ile Glu Arg Asp
 465 470 475
 GAA AAG TAC ATG GCG AGT TTT GAC GAG ATC GTG CCG GAG TTC ATC GAA 1943
 Glu Lys Tyr Met Ala Ser Phe Asp Glu Ile Val Pro Glu Phe Ile Glu
 480 485 490 495
 AAA ATG GAC GAG GCA CTG GCT GAA ATT GGT TTT GTA TTT GGG GAG CAA 1991
 Lys Met Asp Glu Ala Leu Ala Glu Ile Gly Phe Val Phe Gly Glu Gln
 500 505 510

TGG CGA TAG ATCCGGTACC CGAGCACGTG TTGACAATTA ATCATCGGCA	2040
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TAGTATATCG GCATAGTATA ATACGACAAG GTGAGGAACT AAACC ATG AGT ACT	2094
Met Ser Thr	
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GCA CTC GCA ACG CTG GCT GGG AAG CTG GCT GAA CGT GTC GGC ATG GAT	2142
Ala Leu Ala Thr Leu Ala Gly Lys Leu Ala Glu Arg Val Gly Met Asp	
5 10 15	
TCT GTC GAC CCA CAG GAA CTG ATC ACC ACT CTT CGC CAG ACG GCA TTT	2190
Ser Val Asp Pro Gln Glu Leu Ile Thr Thr Leu Arg Gln Thr Ala Phe	
20 25 30 35	
AAA GGT GAT GCC AGC GAT GCG CAG TTC ATC GCA TTA CTG ATC GTT GCC	2238
Lys Gly Asp Ala Ser Asp Ala Gln Phe Ile Ala Leu Leu Ile Val Ala	
40 45 50	
AAC CAG TAC GGC CTT AAT CCG TGG ACG AAA GAA ATT TAC GCC TTT CCT	2286
Asn Gln Tyr Gly Leu Asn Pro Trp Thr Lys Glu Ile Tyr Ala Phe Pro	
55 60 65	
GAT AAG CAG AAT GGC ATC GTT CCG GTG GTG GGC GTT GAT GGC TGG TCC	2334
Asp Lys Gln Asn Gly Ile Val Pro Val Val Gly Val Asp Gly Trp Ser	
70 75 80	
CGC ATC ATC AAT GAA AAC CAG CAG TTT GAT GGC ATG GAC TTT GAG CAG	2382
Arg Ile Ile Asn Glu Asn Gln Phe Asp Gly Met Asp Phe Glu Gln	
85 90 95	
GAC AAT GAA TCC TGT ACA TGC CGG ATT TAC CGC AAG GAC CGT AAT CAT	2430
Asp Asn Glu Ser Cys Thr Cys Arg Ile Tyr Arg Lys Asp Arg Asn His	
100 105 110 115	
CCG ATC TGC GTT ACC GAA TGG ATG GAT GAA TGC CGC CGC GAA CCA TTC	2478
Pro Ile Cys Val Thr Glu Trp Met Asp Glu Cys Arg Arg Glu Pro Phe	
120 125 130	
AAA ACT CGC GAA GGC AGA GAA ATC ACG GGG CCG TGG CAG TCG CAT CCC	2526
Lys Thr Arg Glu Gly Arg Glu Ile Thr Gly Pro Trp Gln Ser His Pro	
135 140 145	
AAA CGG ATG TTA CGT CAT AAA GCC ATG ATT CAG TGT GCC CGT CTG GCC	2574
Lys Arg Met Leu Arg His Lys Ala Met Ile Gln Cys Ala Arg Leu Ala	
150 155 160	
TTC GGA TTT GCT GGT ATC TAT GAC AAG GAT GAA GCC GAG CGC ATT GTC	2622
Phe Gly Phe Ala Gly Ile Tyr Asp Lys Asp Glu Ala Glu Arg Ile Val	
165 170 175	
GAA AAT ACT GCA TAC ACT GCA GAA CGT CAG CCG GAA CGC GAC ATC ACT	2670
Glu Asn Thr Ala Tyr Thr Ala Glu Arg Gln Pro Glu Arg Asp Ile Thr	
180 185 190 195	
CCG GTT AAC GAT GAA ACC ATG CAG GAG ATT AAC ACT CTG CTG ATC GCC	2718
Pro Val Asn Asp Glu Thr Met Gln Glu Ile Asn Thr Leu Leu Ile Ala	
200 205 210	
CTG GAT AAA ACA TGG GAT GAC GAC TTA TTG CCG CTC TGT TCC CAG ATA	2766
Leu Asp Lys Thr Trp Asp Asp Asp Leu Leu Pro Leu Cys Ser Gln Ile	
215 220 225	
TTT CGC CGC GAC ATT CGT GCA TCG TCA GAA CTG ACA CAG GCC GAA GCA	2814
Phe Arg Arg Asp Ile Arg Ala Ser Ser Glu Leu Thr Gln Ala Glu Ala	
230 235 240	

GTA AAA GCT CTT GGA TTC CTG AAA CAG AAA GCC GCA GAG CAG AAG GTG 2862
 Val Lys Ala Leu Gly Phe Leu Lys Gln Lys Ala Ala Glu Gln Lys Val
 245 250 255

GCA GCA TAG ATCTCGAGAA GCTTCCTGCT GAACATCAAA GGCAAGAAAA 2911
 Ala Ala *
 260

CATCTGTTGT CAAAGACAGC ATCCTTGAAC AAGGACAATT AACAGTTAAC AAATAAAAAAC 2971

GCAAAAGAAA ATGCCGATAT CCTATTGGCA TTTTCTTTTA TTTCTTATCA ACATAAAGGT 3031

GAATCCCATATA CCTCGAGCTT CACGCTGCCG CAAGCACTCA GGGCGCAAGG GCTGCTAAAA 3091

GGAAGCGGAA CACGTAGAAA GCCAGTCCGC AGAAACGGTG CTGACCCCGG ATGAATGTCA 3151

GCTACTGGGC TATCTGGACA AGGGAAAACG CAAGCGCAAA GAGAAAGCAG GTAGCTTGCA 3211

GTGGGCTTAC ATGGCGATAG CTAGACTGGG CGGTTTATG GACAGCAAGC GAACCGGAAT 3271

TGCCAGCTGG GGCGCCCTCT GGTAAGGTTG GGAAGCCCTG CAAAGTAAAC TGGATGGCTT 3331

TCTTGCCGCC AAGGATCTGA TGGCGCAGGG GATCAAGATC TGATCAAGAG ACAGGATGAG 3391

GATCGTTTCG C ATG GAT ATT AAT ACT GAA ACT GAG ATC AAG CAA AAG CAT 3441
 Met Asp Ile Asn Thr Glu Thr Glu Ile Lys Gln Lys His
 1 5 10

TCA CTA ACC CCC TTT CCT GTT TTC CTA ATC AGC CCG GCA TTT CGC GGG 3489
 Ser Leu Thr Pro Phe Pro Val Phe Leu Ile Ser Pro Ala Phe Arg Gly
 15 20 25

CGA TAT TTT CAC AGC TAT TTC AGG AGT TCA GCC ATG AAC GCT TAT TAC 3537
 Arg Tyr Phe His Ser Tyr Phe Arg Ser Ser Ala Met Asn Ala Tyr Tyr
 30 35 40 45

ATT CAG GAT CGT CTT GAG GCT CAG AGC TGG GCG CGT CAC TAC CAG CAG 3585
 Ile Gln Asp Arg Leu Glu Ala Gln Ser Trp Ala Arg His Tyr Gln Gln
 50 55 60

CTC GCC CGT GAA GAG AAA GAG GCA GAA CTG GCA GAC GAC ATG GAA AAA 3633
 Leu Ala Arg Glu Glu Lys Glu Ala Glu Leu Ala Asp Asp Met Glu Lys
 65 70 75

GGC CTG CCC CAG CAC CTG TTT GAA TCG CTA TGC ATC GAT CAT TTG CAA 3681
 Gly Leu Pro Gln His Leu Phe Glu Ser Leu Cys Ile Asp His Leu Gln
 80 85 90

CGC CAC GGG GCC AGC AAA AAA TCC ATT ACC CGT GCG TTT GAT GAC GAT 3729
 Arg His Gly Ala Ser Lys Lys Ser Ile Thr Arg Ala Phe Asp Asp Asp
 95 100 105

GTT GAG TTT CAG GAG CGC ATG GCA GAA CAC ATC CGG TAC ATG GTT GAA 3777
 Val Glu Phe Gln Glu Arg Met Ala Glu His Ile Arg Tyr Met Val Glu
 110 115 120 125

ACC ATT GCT CAC CAC CAG GTT GAT ATT GAT TCA GAG GTA TAA 3819
 Thr Ile Ala His His Gln Val Asp Ile Asp Ser Glu Val *
 130 135

AACGAGTAGA AGCTTGCTG TTTTGGCGGA TGAGAGAAGA TTTTCAGCCT GATACAGATT 3879

AAATCAGAAC GCAGAAGCGG TCTGATAAAA CAGAATTTGC CTGGCGGCAG TAGCGCGGTG 3939

GTCCACCTG ACCCATGCC GAACTCAGAA GTGAAACGCC GTAGCGCCGA TGGTAGTGTG 3999

GGGTCTCCCC ATGCGAGAGT AGGGAACTGC CAGGCATCAA ATAAAACGAA AGGCTCAGTC 4059

GAAAGACTGG GCCTTTCGTT TTATCTGTTG TTTGTGCGTG AACGCTCTCC TGAGTAGGAC 4119
AAATCCGCCG GGAGCGGATT TGAACGTTGC GAAGCAACGG CCCGGAGGGT GGCGGGCAGG 4179
ACGCCCCCCA TAAACTGCCA GGCATCAAAT TAAGCAGAAG GCCATCCTGA CGGATGGCCT 4239
TTTTGCGTTT CTACAACTC TTTTGTTTAT TTTTCTAAAT ACATTCAAAT ATGTATCCGC 4299
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GTTACATCGA ACTGGATCTC AACAGCGGTA AGATCCTTGA GAGTTTTTCGC CCCGAAGAAC 4539
GTTTTCCAAT GATGAGCACT TTAAAGTTC TGCTATGTGG CGCGGTATTA TCCCGTGTG 4599
ACGCCGGGCA AGAGCAACTC GGTGCGCGCA TACACTATTC TCAGAATGAC TTGGTTGAGT 4659
ACTCACCAGT CACAGAAAAG CATCTTACGG ATGGCATGAC AGTAAGAGAA TTATGCAGTG 4719
CTGCCATAAC CATGAGTGAT AACACTGCGG CCAACTTACT TCTGACAACG ATCGGAGGAC 4779
CGAAGGAGCT AACCGCTTTT TTGCACAACA TGGGGGATCA TGTAACTCGC CTTGATCGTT 4839
GGGAACCGGA GCTGAATGAA GCCATACCAA ACGACGAGCG TGACACCAGT ATGCCTGTAG 4899
CAATGGCAAC AACGTTGCGC AAATATTAA CTGGCGAACT ACTTACTCTA GCTTCCCGGC 4959
AACAATTAAT AGACTGGATG GAGGCGGATA AAGTTGCAGG ACCACTTCTG CGCTCGGCCC 5019
TTCCGGCTGG CTGTTTATT GCTGATAAAT CTGGAGCCGG TGAGCGTGGG TCTCGCGGTA 5079
TCATTGCAGC ACTGGGGCCA GATGGTAAGC CCTCCCGTAT CGTAGTTATC TACACGACGG 5139
GGAGTCAGGC AACTATGGAT GAACGAAATA GACAGATCGC TGAGATAGGT GCCTCACTGA 5199
TTAAGCATTG GTAAGTGTCA GACCAAGTTT ACTCATATAT ACTTTAGATT GATTTACGCG 5259
CCCTGTAGCG GCGCATTAAG CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA 5319
CTTGCCAGCG CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCTTTTCT CGCCACGTTT 5379
GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT 5439
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CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGAATC 5559
TTGTTCCAAA CTGAACAAC ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG 5619
ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG 5679
AATTTTAACA AAATATTAAC GTTTACAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA 5739
TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTT CACTGAGCGT CAGACCCCGT 5799
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AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC TACCAACTCT 5919
TTTTCCGAAG GTAAGTGGCT TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA 5979
GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGACCCG CCTACATACC TCGCTCTGCT 6039
AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG GGTTGGACTC 6099

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AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT CGTGCACACA 6159
GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG AGCTATGAGA 6219
AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG 6279
AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT 6339
CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTGTGA TGCTCGTCAG GGGGGCGGAG 6399
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TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA 6579
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CCGCATAGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC GCCCTGACGG 6699
GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG 6759
TGTCAGAGGT TTTACCGTC ATCACCGAAA CGCGCGAGGC AGCAAGGAGA TGGCGCCCAA 6819
CAGTCCCCCG GCCACGGGGC CTGCCACCAT ACCCAGCCCG AAACAAGCGC TCATGAGCCC 6879
GAAGTGGCGA GCCCGATCTT CCCCATCGGT GATGTCGGCG ATATAGGCGC CAGCAACCGC 6939
ACCTGTGGCG CCGGTGATGC CGGCCACGAT GCGTCCGGCG TAGAGGATCT GCTCATGTTT 6999
GACAGCTTAT C 7010

```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 227 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```

Met Thr Pro Asp Ile Ile Leu Gln Arg Thr Gly Ile Asp Val Arg Ala
 1             5             10             15
Val Glu Gln Gly Asp Asp Ala Trp His Lys Leu Arg Leu Gly Val Ile
          20             25             30
Thr Ala Ser Glu Val His Asn Val Ile Ala Lys Pro Arg Ser Gly Lys
      35             40             45
Lys Trp Pro Asp Met Lys Met Ser Tyr Phe His Thr Leu Leu Ala Glu
      50             55             60
Val Cys Thr Gly Val Ala Pro Glu Val Asn Ala Lys Ala Leu Ala Trp
      65             70             75             80
Gly Lys Gln Tyr Glu Asn Asp Ala Arg Thr Leu Phe Glu Phe Thr Ser
          85             90             95
Gly Val Asn Val Thr Glu Ser Pro Ile Ile Tyr Arg Asp Glu Ser Met
          100             105             110
Arg Thr Ala Cys Ser Pro Asp Gly Leu Cys Ser Asp Gly Asn Gly Leu
          115             120             125

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Glu Leu Lys Cys Pro Phe Thr Ser Arg Asp Phe Met Lys Phe Arg Leu
 130 135 140
 Gly Gly Phe Glu Ala Ile Lys Ser Ala Tyr Met Ala Gln Val Gln Tyr
 145 150 155 160
 Ser Met Trp Val Thr Arg Lys Asn Ala Trp Tyr Phe Ala Asn Tyr Asp
 165 170 175
 Pro Arg Met Lys Arg Glu Gly Leu His Tyr Val Val Ile Glu Arg Asp
 180 185 190
 Glu Lys Tyr Met Ala Ser Phe Asp Glu Ile Val Pro Glu Phe Ile Glu
 195 200 205
 Lys Met Asp Glu Ala Leu Ala Glu Ile Gly Phe Val Phe Gly Glu Gln
 210 215 220
 Trp Arg *
 225

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 262 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Met Ser Thr Ala Leu Ala Thr Leu Ala Gly Lys Leu Ala Glu Arg Val
 1 5 10 15
 Gly Met Asp Ser Val Asp Pro Gln Glu Leu Ile Thr Thr Leu Arg Gln
 20 25 30
 Thr Ala Phe Lys Gly Asp Ala Ser Asp Ala Gln Phe Ile Ala Leu Leu
 35 40 45
 Ile Val Ala Asn Gln Tyr Gly Leu Asn Pro Trp Thr Lys Glu Ile Tyr
 50 55 60
 Ala Phe Pro Asp Lys Gln Asn Gly Ile Val Pro Val Val Gly Val Asp
 65 70 75 80
 Gly Trp Ser Arg Ile Ile Asn Glu Asn Gln Gln Phe Asp Gly Met Asp
 85 90 95
 Phe Glu Gln Asp Asn Glu Ser Cys Thr Cys Arg Ile Tyr Arg Lys Asp
 100 105 110
 Arg Asn His Pro Ile Cys Val Thr Glu Trp Met Asp Glu Cys Arg Arg
 115 120 125
 Glu Pro Phe Lys Thr Arg Glu Gly Arg Glu Ile Thr Gly Pro Trp Gln
 130 135 140
 Ser His Pro Lys Arg Met Leu Arg His Lys Ala Met Ile Gln Cys Ala
 145 150 155 160
 Arg Leu Ala Phe Gly Phe Ala Gly Ile Tyr Asp Lys Asp Glu Ala Glu
 165 170 175

Arg Ile Val Glu Asn Thr Ala Tyr Thr Ala Glu Arg Gln Pro Glu Arg
 180 185 190
 Asp Ile Thr Pro Val Asn Asp Glu Thr Met Gln Glu Ile Asn Thr Leu
 195 200 205
 Leu Ile Ala Leu Asp Lys Thr Trp Asp Asp Asp Leu Leu Pro Leu Cys
 210 215 220
 Ser Gln Ile Phe Arg Arg Asp Ile Arg Ala Ser Ser Glu Leu Thr Gln
 225 230 235 240
 Ala Glu Ala Val Lys Ala Leu Gly Phe Leu Lys Gln Lys Ala Ala Glu
 245 250 255
 Gln Lys Val Ala Ala *
 260

(2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 139 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Met Asp Ile Asn Thr Glu Thr Glu Ile Lys Gln Lys His Ser Leu Thr
 1 5 10 15
 Pro Phe Pro Val Phe Leu Ile Ser Pro Ala Phe Arg Gly Arg Tyr Phe
 20 25 30
 His Ser Tyr Phe Arg Ser Ser Ala Met Asn Ala Tyr Tyr Ile Gln Asp
 35 40 45
 Arg Leu Glu Ala Gln Ser Trp Ala Arg His Tyr Gln Gln Leu Ala Arg
 50 55 60
 Glu Glu Lys Glu Ala Glu Leu Ala Asp Asp Met Glu Lys Gly Leu Pro
 65 70 75 80
 Gln His Leu Phe Glu Ser Leu Cys Ile Asp His Leu Gln Arg His Gly
 85 90 95
 Ala Ser Lys Lys Ser Ile Thr Arg Ala Phe Asp Asp Asp Val Glu Phe
 100 105 110
 Gln Glu Arg Met Ala Glu His Ile Arg Tyr Met Val Glu Thr Ile Ala
 115 120 125
 His His Gln Val Asp Ile Asp Ser Glu Val *
 130 135

Table 1: Sequences of Oligos for PCR

Figure 3ab

left: TGACCCCTCACAAGGAGACGACCTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA

right: TACAAATGTGGTATGGCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCCCCGCTTTCCA

template: pJP5603

targeting vector: pSV-paz11

Figure 3c

a-left: CTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA

a-right: ATGATCCTCTAGAGTCGGTGCTCACTGCCCCGCTTTCCA

b-left: AGACGACCTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA

b-right: GCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCCCCGCTTTCCA

c-left: CACAAGGAGACGACCTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA

c-right: TGGTATGGCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCCCCGCTTTCCA

d-left: TGACCCCTCACAAGGAGACGACCTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA

d-right: TACAAATGTGGTATGGCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCCCCGCTTTCCA

e-left:

CACGCCCCTGACCCCTCACAAGGAGACGACCTTCCATGACCGAGTACAAGAGGGATGTAACGCACTGA

e-right:

TAAACCTCTACAAATGTGGTATGGCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCCCCGCTTTCCA

f-left:

TCCCCTGACCCACGCCCCTGACCCCTCACAAGGAGACGACCTTCCATGACCGAGTACAAGAGGGATGT
AACGCACTGA

f-right:

TAAAGCAAGTAAACCTCTACAAATGTGGTATGGCTGATTATGATCCTCTAGAGTCGGTGCTCACTGCC
CGCTTTCCA

template: pJP5603

targeting vector: pSV-paz11

Figure 3d

a-left:

TCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGTGCAAGGCTGCTAAAGGAA

a-right:

TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAAGTCCAGCAT

b-left:

CACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCAGACGATGGCAAGGCTGCTAAAGGAA

b-right:

TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAAGTCCAGCAT

c-left:

TTAACCGTCACGAGCATCATCCTCTGCATGGTCAGGTCATGGATGAGCACAAGGCTGCTAAAGGAA

c-right:

TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAAGTCCAGCAT

d-left:

TGCTGCTGAACGGCAAGCCGTTGCTGATTCGAGGCGTTAACCGTCACGACAAGGCTGCTAAAGGAA

d-right:

TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAAGTCCAGCAT

e-left:

TCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTCAAGGCTGCTAAAGGAA

e-right:

TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAAGTCCAGCAT

f-left:

TGGAGTGACGGCAGTTATCTGGAAGATCAGGATATGTGGCGGATGAGCGCAAGGCTGCTAAAGGAA

f-right:

TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAACTCCAGCAT

g-left:

TGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCAAGGGCTGCTAAAGGAA

g-right:

TAATGCGAACAGCGCACGGCGTTAAAGTTGTTCTGCTTCATCAGCAGGATGGCGAAGAACTCCAGCAT

h-left:

TGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCAAGGGCTGCTAAAGGAA

h-right:

TATTTTGTGACACCAGACCAACTGGTAATGGTAGCGACCGGCGCTCAGCTGGCGAAGAACTCCAGCAT

template: pJP5603

targeting vector: pSV-paz11

Figure 4

left:

TCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAAAGCTTATGCCCACCAGC
TGGTATGGCTGATTATGATC

right:

TCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACA
ATCTACCACCAGCTCTTTTCTACGGGGTCTGACGC

template: pBR322

targeting vector: Hoxa-P1

Figure 5

left:

TGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTTAATACGACTCACTATAGGGAGAACA
GGAAACAGCTATGCCCATAACCCAGAGTA

right:

TGCGCCGCTACAGGGCGCGTCCATTGCGCCATTCAGGCCTGACTCACTAGTGATGGTGATGGTGATGTGG
GGGGTGCCGCTCAGT

template: pmtrx (a pBluescript vector carrying mouse trithorax cDNA)

targeting vector: pZero2.1

Figure 6

left:

TGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGGAGAAAAAATCACT
GGATATACCACCG

right:

TACAGGGCGCGTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACGCCCCGCCCTGC
CACTCATCGCA

template: pMAK705

targeting vector: pBAD-24 backbone Amp resistant gene

Figure 8

i:

TGCCAAGCTTGACCCACTGTGGAAGTGTTCCAAAAAGCGGGAAGGCTCTTGAGCTACTTCACTAACAAC
CGG

g:

TCACCATCTTCGGGCCATTTGTAGACTGGAATATTTGAGCTATGAGTGTGCTACTTCACTAACAACCG
G

h:

TGGCCCCAGGGTGACGCGGACATGGAGTTGTGCCAGGGCACTGGTCCATGAGAGTGCCAAGCTACTC
GCGAC

template: pKaZ

targeting vector: Hoxa-P1

Figure 9

j:

TAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCT
TTGCCCTGGTTTATAACTTCGTATAGCATACATTATACGAAGTTATGGGCTGCTAAAGGAAGCGGAACAC
G

k:

TGGCAGTTCAGGCCAATCCGCGCCGGATGCGGTGTATCGCTCGCCACTTCAACATCAACGGTAATCGCC
ATTTGACCATATAACTTCGTATAATGTATGCTATACGAAGTTATCCCCAGAGTCCCGCTCAGAAGAACT
template: pJP5603

targeting vector: JC9604 chromosome

Figure 10

l:

TAGCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACCCATCAC
ATATACCTGCCGTTCACTAT

m:

TATCGGTGGCCGTGGTGTGCGGCTCCGCCGCCTTCATACTGCACCGGGCGGGAAGGCGATTCCGAAGCCC
AACCTTTCATAGAAGCC

template: pIB279

targeting vector: pSV-paX1

l*: GCTTGGCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAA

m*:

TCGGTGGCCGTGGTGTGCGGCTCCGCCGCCTTCATACTGCACCGGGCGGGAAGGATCCACAGATTTGATC
CAGCGATACAGC

template: pSV-paz11

targeting vector: pSV-sacB-neo

Figure 11

n:

TACCGCATTAAAGCTTATCGATGATAAGCTGTCAAACATGAGAATTGACCCGGAACCCTTCTCGAGGAA
GTTCTATTCTCTAGAAAGTATAGGAACCTCCGAATAAATACCTGTGACGGAAGATCACTT

p:

TTCCCTCAAGAATTTTACTCTGTCAGAAACGGCCTTAACGACGTAGTCGAGGGACCTAGAAGTTCCTAT
ACTTTCTAGAGAATAGGAACCTCATTATCACTTATTCAGGCGTAGCACCAGGCG

template: pMAK705

targeting vector: Hoxa-P1

Figure 12

left:

TGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGGAGAAAAAAATCACT
GGATATACCACCG

right:

TACAGGGCGCGTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACGCCCCGCCCTGC
CACTCATCGCA

template: pMAK705

targeting vector: pBAD-24 backbone Amp resistant gene





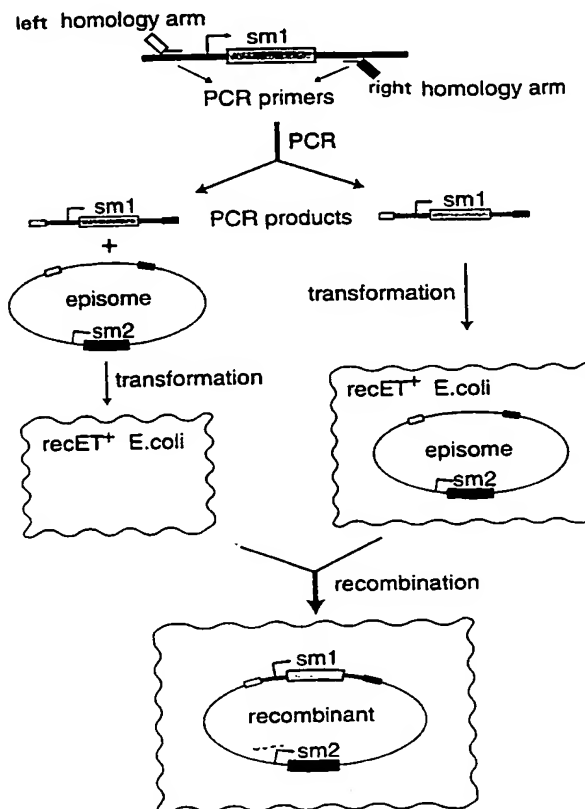
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(71) Applicant (for all designated States except US): EUROPAISCHES LABORATORIUM FÜR MOLEKULARBIOLOGIE (EMBL) [DE/DE]; Meyerhofstrasse 1, D-69117 Heidelberg (DE).			
(72) Inventors; and (75) Inventors/Applicants (for US only): STEWART, Francis [AU/DE]; Lärchenweg 3, D-69181 Leimen (DE). ZHANG, Youming [CN/DE]; Friedrich-Ebert-Anlage 51e, D-69117 Heidelberg (DE). BUCHHOLZ, Frank [DE/DE]; Neuenkirchener Weg 44a, D-28779 Bremen (DE).		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
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(54) Title: NOVEL DNA CLONING METHOD RELYING ON THE E. COLI RECE/RECT RECOMBINATION SYSTEM

(57) Abstract

The invention refers to a novel method for cloning DNA molecules using a homologous recombination mechanism between at least two DNA molecules comprising: a) providing a host cell capable of performing homologous recombination, b) contacting in said host cell a first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred. In particular, it relies on the use of the E. coli RecE and RecT proteins, the bacteriophage Red-alpha and Red-beta proteins, or the phage P22 recombination system. The beneficial effects of concomitant expression of the RecBC inhibitor genes (e.g. Red-Gamma) is also exemplified.



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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OLINER J.D. ET AL.: "In vivo Cloning of PCR Products in E. coli" NUCLEIC ACIDS RESEARCH, vol. 21, no. 22, 1993, pages 5192-5197, XP002064297 cited in the application	1-6, 8-13, 19, 20, 22-27, 34, 35, 37-42, 46-48, 50 18, 28
Y	See page 5192, column 2, line 8 - line 31, and discussion section	
Y	NUSSBAUM A ET AL: "Restriction-stimulated homologous recombination of plasmids by the RecE pathway of Escherichia coli" GENETICS, vol. 130, no. 1, January 1992, pages 37-49, XP002103370 see figures 1,7	18, 28
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☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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National Application No

PCT/EP 98/07945

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>ZHANG Y ET AL: "A new logic for DNA engineering using recombination in Escherichia coli" NATURE GENETICS, vol. 20, no. 2, October 1998, pages 123-128, XP002103371 see the whole document ---</p>	1-50
A	<p>KOLODNER R ET AL: "Homologous pairing proteins encoded by the Escherichia coli recE and recT genes" MOLECULAR MICROBIOLOGY, vol. 11, no. 1, 1994, pages 23-30, XP002064301 cited in the application ---</p>	
A	<p>LUISI-DELUCA C ET AL: "Genetic and physical analysis of plasmid recombination in recB recC sbcB and recB recC sbcA Escherichia coli K-12 mutants" GENETICS, vol. 122, 1989, pages 269-278, XP002064305 ---</p>	
A	<p>DEGRYSE E: "Evaluation of Escherichia coli recBC sbcBC mutants for cloning by recombination in vivo" JOURNAL OF BIOTECHNOLOGY, vol. 2, no. 39, 15 April 1995, page 181-187 XP004036984 ---</p>	
A	<p>YANG X- ET AL: "Homologous recombination based modification in Escherichia coli and germ line transmission in transgenic mice of a bacterial artificial chromosome" NATURE BIOTECHNOLOGY, vol. 15, September 1997, pages 859-865, XP002103372 ---</p>	
A	<p>MURPHY K: "Lambda Gam protein inhibits the helicase and Chi-stimulated recombination activities of Escherichia coli RecBCD enzyme" JOURNAL OF BACTERIOLOGY, vol. 173, no. 18, September 1991, pages 5808-5821, XP002103373 -----</p>	



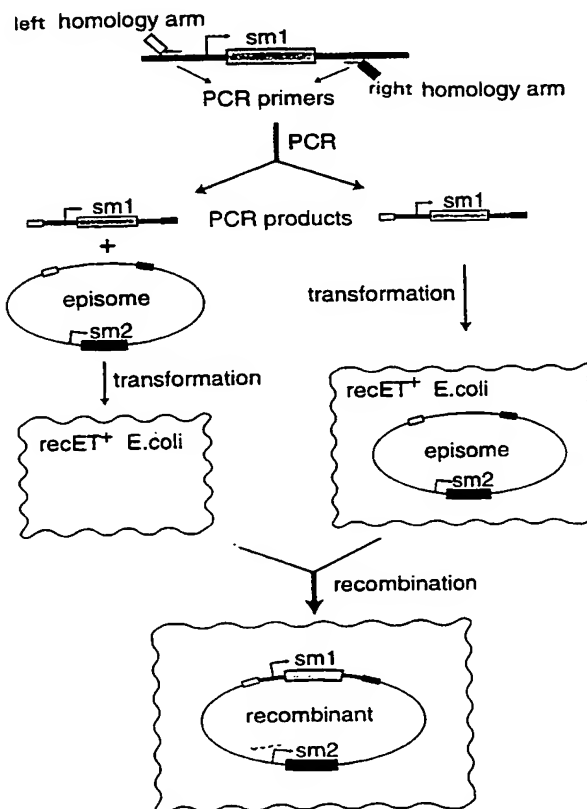
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(54) Title: NOVEL DNA CLONING METHOD RELYING ON THE E. COLI RECE/RECT RECOMBINATION SYSTEM

(57) Abstract

The invention refers to a novel method for cloning DNA molecules using a homologous recombination mechanism between at least two DNA molecules comprising: a) providing a host cell capable of performing homologous recombination, b) contacting in said host cell a first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred. In particular, it relies on the use of the E. coli RecE and RecT proteins, the bacteriophage Red-alpha and Red-beta proteins, or the phage P22 recombination system. The beneficial effects of concomitant expression of the RecBC inhibitor genes (e.g. Red-Gamma) is also exemplified.



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AMENDED CLAIMS

[received by the International Bureau on 04 August 1999 (04.08.99);
original claims 1-50 replaced by new claims 1-64 (10 pages)]

1. A method for cloning DNA molecules in procaryotic cells comprising the steps of:
 - a) providing a procaryotic host cell capable of performing homologous recombination,
 - b) contacting in said host cell a circular first DNA molecule which is capable of being replicated in said host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and
 - c) selecting a host cell in which homologous recombination between said first and second DNA molecules has occurred.
2. The method according to claim 1 wherein the homologous recombination occurs via the recET cloning mechanism.
3. The method according to claim 2 wherein the host cell is capable of expressing recE and recT genes.
4. The method according to claim 3 wherein the recE and recT genes are selected from E.coli recE and recT genes or from λ red α and red β genes.
5. The method according to claim 3 or 4 wherein the host cell is transformed with at least one vector capable of expressing recE and/or recT genes.
6. The method of claim 3, 4 or 5 wherein the expression of the recE and/or recT genes is under control of a regulatable promoter.

7. The method of claim 5 or 6 wherein the recT gene is overexpressed versus the recE gene.
8. The method according to any one of claims 3 to 7 wherein the recE gene is selected from a nucleic acid molecule comprising
 - (a) the nucleic acid sequence from position 1320 (ATG) to 2159 (GAC) as depicted in Fig.7B,
 - (b) the nucleic acid sequence from position 1320 (ATG) to 1998 (CGA) as depicted in Fig.13B,
 - (c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
 - (d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequence from (a), (b) and/or (c).
9. The method according to any one of claims 3 to 8 wherein the recT gene is selected from a nucleic acid molecule comprising
 - (a) the nucleic acid sequence from position 2155 (ATG) to 2961 (GAA) as depicted in Fig.7B,
 - (b) the nucleic acid sequence from position 2086 (ATG) to 2868 (GCA) as depicted in Fig.13B,
 - (c) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
 - (d) a nucleic acid sequence which hybridizes under stringent conditions with the nucleic acid sequences from (a), (b) and/or (c).
10. The method according to any one of the previous claims wherein the host cell is a gram-negative bacterial cell.
11. The method according to claim 10 wherein the host cell is an Escherichia coli cell.

12. The method according to claim 11 wherein the host cell is an *Escherichia coli* K12 strain.
13. The method according to claim 12 wherein the *E.coli* strain is selected from JC 8679 and JC 9604.
14. The method according to any one of the previous claims wherein the host cell further is capable of expressing a *recBC* inhibitor gene.
15. The method according to claim 14 wherein the host cell is transformed with a vector expressing the *recBC* inhibitor gene.
16. The method according to claim 14 or 15 wherein the *recBC* inhibitor gene is selected from a nucleic acid molecule comprising
 - (a) the nucleic acid sequence from position 3588 (ATG) to 4002 (GTA) as depicted in Fig.13B,
 - (b) a nucleic acid encoding the same polypeptide within the degeneracy of the genetic code and/or
 - (c) a nucleic acid sequence which hybridizes under stringent conditions (as defined above) with the nucleic acid sequence from (a) and/ or (b).
17. The method according to any one of claims 13 to 16 wherein the host cell is a prokaryotic *recBC* + cell.
18. The method according to any one of the previous claims wherein the first DNA molecule is an extrachromosomal DNA molecule containing an origin of replication which is operative in the host cell.
19. The method according to claim 18 wherein the first DNA molecule is selected from plasmids, cosmids, P1 vectors, BAC vectors and PAC vectors.

20. The method according to any one of claims 1-18 wherein the first DNA molecule is a host cell chromosome.
21. The method according to any one of the previous claims wherein the second DNA molecule is linear.
22. The method according to any one of the previous claims wherein the regions of sequence homology are at least 15 nucleotides each.
23. The method according to one of claims 1 to 16 wherein the second DNA molecule is obtained by an amplification reaction.
24. The method according to one of the previous claims wherein the first and/or second DNA molecules are introduced into the host cells by transformation.
25. The method according to claim 24 wherein the transformation method is electroporation.
26. The method according to one of claims 1 to 25 wherein the first and second DNA molecules are introduced into the host cell simultaneously by co-transformation.
27. The method according to one of claims 1 to 25 wherein the second DNA molecule is introduced into a host cell in which the first DNA molecule is already present.
28. The method according to one of the previous claims wherein the second DNA molecule contains at least one marker gene placed between the two regions of sequence homology and wherein homologous recombination is detected by expression of said marker gene.

29. The method according to claim 28 wherein gene presence is selected from antibiotic resistance genes, deficiency complementation genes and reporter genes.
30. The method of any one of claims 1 to 29 wherein the first DNA molecule contains at least one marker gene between the two regions of sequence homology and wherein homologous recombination is detected by lack of expression of said marker gene.
31. The method of any one of claims 1 to 30 wherein said marker gene is selected from genes which, under selected conditions, convey a toxic or bacteriostatic effect on the cell, and reporter genes.
32. A method according to any one of the previous claims wherein the first DNA molecule contains at least one target site for a site specific recombinase between the two regions of sequence homology and wherein homologous recombination is detected by removal of said target site.
33. A method for cloning DNA molecules comprising the steps of:
 - (a) providing a source of RecE and RecT proteins,
 - (b) contacting a first DNA molecule which is capable of being replicated in a suitable host cell with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, under conditions which favour homologous recombination between said first and second DNA molecules and
 - (c) selecting DNA molecules in which homologous recombination between said first and second DNA molecules has occurred.
34. The method of claim 33 wherein said RecE and RecT or proteins are selected from E.coli RecE and RecT proteins or from phage λ Red α and Red β proteins.

35. The method of claim 33 or 34 wherein the recombination occurs in vitro.
36. The method of claim 33 or 34 wherein the recombination occurs in vivo.
37. A method for making a recombinant DNA molecule comprising introducing into a prokaryotic host cell a circular first DNA molecule which is capable of being replicated in said host cell, and introducing a second DNA molecule comprising a first and a second region of sequence homology to a third and fourth region, respectively, on the first DNA molecule, said host cell being capable of performing homologous recombination, such that a recombinant DNA molecule is made, said recombinant DNA molecule comprising the first DNA molecule wherein the sequences between said third and fourth regions have been replaced by sequences between the first and second regions of the second DNA molecule.
38. The method according to claim 37 which further comprises detecting the recombinant DNA molecule.
39. A method for making a recombinant DNA molecule comprising introducing into a prokaryotic host cell, containing a chromosomal first DNA molecule, a second DNA molecule comprising a first and a second region of sequence homology to a third and a fourth region, respectively, on the host chromosomal first DNA molecule, said host cell being capable of performing homologous recombination, such that a recombinant DNA molecule is made, said recombinant DNA molecule comprising the chromosomal first DNA molecule wherein the sequences between said third and fourth regions have been replaced by sequences between the first and second regions of the second DNA molecule.

40. The method according to claim 39 which further comprises detecting the recombinant DNA molecule.
41. The method according to any one of claims 37 to 40, wherein the host cell is capable of expressing RecE and RecT proteins or λ exo and $\lambda\beta$ proteins.
42. A method for cloning DNA molecules comprising the steps of:
- (a) contacting in vitro a first DNA molecule with a second DNA molecule comprising at least two regions of sequence homology to regions on the first DNA molecule, in the presence of RecE and RecT proteins and under conditions which favour homologous recombination between said first and second DNA molecules; and
 - (b) selecting a DNA molecule in which homologous recombination between said first and second DNA molecules has occurred.
43. A method for making a recombinant DNA molecule comprising contacting in vitro a first DNA molecule with a second DNA molecule comprising a first and a second region of sequence homology to a third and a fourth region on the first DNA molecule, in the presence of RecE and RecT proteins and under conditions in which homologous recombination can occur, such that a recombinant DNA molecule is made, said recombinant DNA molecule comprising the first DNA molecule wherein the sequences between said third and fourth regions have been replaced by sequences between the first and second regions of the second DNA molecule.
44. The method of claim 42, which further comprises between steps (a) and (b) the step of introducing the product step (a) into a cell, wherein recombination occurs in the cell.

45. Use of cells capable of expressing the recE and recT genes as a host cell for a cloning method involving homologous recombination.
46. Use of a vector system capable of expressing recE and recT genes in a host cell for a cloning method involving homologous recombination.
47. Use of claims 45 or 46 wherein the recE and recT genes are selected from E.coli recE and recT genes or from λ red α and red β genes.
48. Use of a source of RecE and RecT proteins for a cloning method involving homologous recombination.
49. Use of claim 48 wherein said RecE and RecT or proteins are selected from E.coli RecE and RecT proteins or from phage λ Red α and Red β proteins.
50. A reagent kit for cloning comprising
 - (a) a host cell
 - (b) means of expressing recE and recT genes in said host cell and
 - (c) a recipient cloning vehicle capable of being replicated in said cell.
51. The reagent kit according to claim 50 wherein the means (b) comprise a vector system capable of expressing the recE and recT genes in the host cell.
52. The reagent kit according to claim 50 or 51 wherein the recE and recT genes are selected from E.coli recE and recT genes or from λ red α and red β genes.
53. A reagent kit for cloning comprising
 - (a) a source for RecE and RecT proteins and

(b) a recipient cloning vehicle capable of being propagated in a host cell.

54. The reagent kit according to claim 53 further comprising a host cell suitable for propagating said recipient cloning vehicle.
55. The reagent kit according to claim 53 or 54 wherein said RecE and RecT or proteins are selected from E.coli RecE and RecT proteins or from phage λ Red α and Red β proteins.
56. The reagent kit according to any one of claims 50-55 further comprising means for expressing a site specific recombinase in said host cell.
57. The reagent kit according to any one of claims 50-56 further comprising nucleic acid amplification primers comprising a region of homology to said recipient cloning vehicle.
58. A reagent kit for cloning comprising first and second DNA amplification primers and a recipient cloning vehicle that is a circular DNA molecule, said first DNA amplification primer having a first region of sequence homology to a third region on the circular recipient cloning vehicle, and said second DNA amplification primer having a second region of sequence homology to a fourth region on the circular recipient cloning vehicle.
59. The reagent kit of claim 58, further comprising a prokaryotic host cell that is capable of performing homologous recombination.
60. The reagent kit of claim 58 or 59, further comprising a means of expressing RecE and RecT proteins or Red α and Red β proteins.

61. The reagent kit according to any one of claims 58-60, wherein the means comprises a vector system capable of expressing the *recE* and *recT* genes in the host cell.
62. The reagent kit according to any one of claims 58-61, further comprising a phenotypic marker located in the recipient cloning vehicle between the third and fourth regions of sequence homology.
63. The reagent kit according to any one of claims 58-62, wherein the recipient cloning vehicle further comprises a recognition site for a site-specific recombinase on the recipient cloning vehicle between the third and fourth regions of sequence homology.
64. The reagent kit of claim 63, further comprising means for expressing a site-specific recombinase in said host cell.